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RA antigenic peptides

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RA antigenic peptides

The present invention provides novel naturally-processed RA antigenic peptides which are candidate markers for erosive and non-erosive RA. These antigenic peptides are presented by human MHC class II HLA-DR molecules. Moreover, these antigenic peptides linked to MHC class II molecules, as well as antibodies reactive with said antigenic peptides, nucleic acids encoding said antigenic peptides, and nucleic acid constructs and host cells for expressing said antigenic peptides are provided. The antigenic peptides of the invention as well as the polypeptides they are derived from can be used as markers in diagnosis of RA and in therapy as anti-RA vaccines.

Rheumatoid Arthritis (RA), originally termed chronic polyarthritis, is a systemic autoimmune disease and one of the most debilitating forms of articular inflammation (Feldmann, M. et al., Cell 85 (1996) 307-310; Dedhia, H.V. & DiBartolomeo, A., Critical care clinics 18 (2002) 841-854). Typically, RA causes joint pain, deformities and severe joint stiffness. The disease can also have its manifestation outside the joints, especially in patients who are positive for an autoantibody, termed "rheumatoid factor" (RF) (Mageed, R.A., in: van Venrooij, W.J. & Maini, R.N. eds., Manual of biological markers of disease, Kluwer Academic Publishers (1996) 1-18). RA occurs quite frequently in the Caucasian population with the susceptibility to RA being influenced by genetic and environmental factors. Both have a crucial effect on the onset and the progression of this autoimmune disease. Approximately 4% of the total population has an increased genetic susceptibility to RA, roughly 20% of which (around 1% of the total population) develops RA as a result of, as yet, uncharacterized non-inheritable factors. Beyond that, RA shows a significant bias in the sex ratio: women have a three fold higher risk for RA than men, indicating that sex hormones may also be involved in the pathogenesis.

In the beginning, RA progresses slowly. Typical early stage symptoms are palm sweating, morning stiffness of fingers and symmetrical joint inflammation (www.medicine-worldwide.de). In addition, rheumatoid nodules can appear which is an indication for tissue affection outside the joints. In a simplified model, the immune system produces autoantibodies against healthy tissue (www.netdoktor.de). These autoantibodies attack the articular cartilage in the joint leading to its inflammation and later on to its destruction. This destruction stimulates the immune system to produce more autoantibodies. In addition, cytokines like tumor necrosis-factor alpha (TNF-α) and Interleukin-1 (IL-1) are produced which enhance the inflammatory reaction even further (Houssiau, F.A., Clin Rheumatol 14 Suppl 2 (1995) 10-13). The synovium begins to swell due to infiltration of additional cells of the immune system, such as macrophages and T cells. These cells are actively involved in causing further cell death and in driving joint inflammation (Fox, D.A., Arthritis Rheum 40 (1997) 598-609; Choy, E.H. & Panayi, G.S., N Engl J Med 344 (2001) 907-916). This process resembles a vicious circle of autoantibody production, joint inflammation and joint destruction.

Typically, RA progresses chronically, with 85-90% of all RA patients showing a mild to moderate disease development. Aggressive disease forms leading to complete loss of joint function up to the degree of invalidity is experienced by 10-15% of the patients. In this advanced RA state, patients have a permanent articular inflammation and display rheumatoid nodules. They suffer from strong chronical pain and the inflammation leads to severe finger stiffness and irreversible joint deformations or dislocations.

Diagnosis

There is growing evidence that therapeutic intervention early in the disease can reduce the extent of joint damage (Egsmose, C. et al., J Rheumatol 22 (1995) 2208-2213; Van der Heide, A. et al., Ann Intern Med 124 (1996) 699-707). Since treatment with disease-modifying antirheumatic drugs (DMARDs) is only justified when the risk:benefit or cost:effectiveness ratios are favorable, it is mandatory to be able to differentiate between RA and other forms of arthritis shortly after onset of the disease (Kirwan, J.R. & Quilty, B., Clin Exp Rheumatol 15 (1997) 15-25). The diagnosis is made by established criteria based on clinical history, physical examination and laboratory tests. The American Society of Rheumatism published a catalog of criteria to help gaining objective evidence for RA (Arnett, F.C. et al., Arthritis Rheum 31 (1987) 315-324). But so far, not a single test is available which is specific for RA. Several biological and biochemical markers, e.g. C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), antinuclear antibody (ANA) or RF are utilized for the evaluation of RA. However, these markers are non-specific, as they appear in other inflammatory or autoimmune diseases as well. The RF, for instance, is

an autoantibody that is present in the serum of approximately 50% of RA patients. Since increased levels of the same autoantibody can also be found in the context of other inflammatory diseases, such as Sjögren syndrome, endokarditis or chronical hepatitis, RF is unsuitable to serve as a diagnostic marker for RA. Rather than being of diagnostic value *per se*, the above mentioned biochemical and biological markers are useful for assessing disease activity and prognosis as well as in the treatment and management of RA patients (Nakamura, R.M., J Clin Lab Anal 14 (2000) 305-313).

Recently, a diagnostic set of criteria was developed that consists of clinical and biochemical aspects which were claimed to discriminate, at an early state, between self-limiting, persistent non-erosive, and persistent erosive RA (Visser, H. et al., Arthritis Rheum 46 (2002) 357-365). Self-limiting arthritis was characterized by natural remission: there was no arthritis on examination in a patient for a certain period of time. Erosive arthritis was defined based on the presence of erosions on radiographs of the hands and/or feet. In particular, the use of antibodies recognizing cyclic citrullinated peptides appears to be promising and suggests an important role for citrullinated antigens in the early diagnosis and prognosis of erosive RA (Schellekens, G.A. et al., J Clin Invest 101 (1998) 273-281; Vincent, C. et al., J Rheumatol 25 (1998) 838-846). The early recognition of erosive RA allows early intervention with DMARDs, which will lead to earlier disease control and improvement of disease outcome (Symmons, D.P.M. et al., J Rheumatol 25 (1998) 1072-1077; Anderson, J.J. et al., Arthritis Rheum 43 (2000) 22-29). Likewise, early recognition of self-limiting and non-erosive arthritis will prevent unnecessary treatment with potentially toxic therapeutics (Fries, J.F. et al., Arthritis Rheum 36 (1993) 297-306.

Therapy

The goal of any anti-rheumatic therapy is to relieve pain in order to ease the activities of every day life. So far, complete healing of RA is not possible, but by applying modern therapies the progression of the disease can be slowed down or even stopped. Due to individual differences, each patient requires an individualized therapy and early diagnosis, as mentioned before, is desirable. RA therapy is complex and includes lifelong medicinal treatment as well as physio- and radiotherapy. DMARDs used in RA therapy are basic therapeutics (e.g. Methotrexate, Sulfasalazin, Hydroxychloroquin, Leflunomid, Azathioprin), cortisone, non-steroidal anti-inflammatory drugs (NSAID) or monoclonal antibodies against the pro-inflammatory cytokines TNF-α, IL-1β or their respective receptors (http://rheuma-online.de). These drugs have all in common that they are inhibitors of inflammation by suppressing the immune response. The main disadvantage is their lack of specificity for RA, their adverse effects and their inability to effectively target the causes of RA.

Autoimmunity

Autoimmunity starts when a specific adaptive immune response is initiated against self antigens (autoantigens) manifested by the development of self-reactive T or B cells. The normal consequence of an adaptive immune response against a foreign antigen is the clearance of the antigen from the body. When an adaptive immune response develops against a self antigen, however, the antigen can in most cases not be completely removed from the body, leading to a sustained immune response. As a consequence, the effector mechanisms of immunity cause chronic inflammatory injury to tissues. The mechanisms of tissue damage are essentially the same in autoimmune disease as those that operate in protective immunity and in hypersensitivity. Even though it is not well understood what triggers autoimmunity, several events which are nowadays believed to contribute to the induction of autoimmune diseases and selection of autoantigenic targets have been summarized most recently (Marrack, P. et al., Nat Med 7 (2001) 899-905).

Autoimmune diseases are controlled by properties of particular genes of each individual and environmental factor. The host's genes affect the susceptibility to autoimmunity at least at three levels. First, some of the genes affect the overall reactivity of the immune system and, thus, can predispose the individual to certain or to several different types of autoimmune diseases. Second, this altered immunoreactivity is funneled to particular autoantigens and tissues by other genes that affect recognition of antigenic peptides by T cells. Third, still other genes act on the ability of target tissues to modulate immune attack for instance by influencing the activity of effector cells of the immune system which are destined to initiate an autoaggressive attack. The latter two sets of genes dictate which antigens will be the targets of autoimmunity and hence which organs will be attacked and what damage will occur.

In addition, signals from the environment influence the development of autoimmunity at the same three levels, by affecting the overall reactivity of the immune system, the antigen-specificity and the state of the potential target tissue. And finally, there is cross-talk between genetic and environmental factors.

Major histocompatibility complex (MHC)

Population studies, genotyping and modern approaches at the molecular level have unanimously shown that certain genes encoded by the major histocompatibility complex (MHC) confer a significantly higher risk for the development of RA (Stastny, P., Tissue Antigens 4 (1974) 571-579; Wordsworth, P. et al., PNAS 86 (1989) 10049-10053; Wordsworth, P. & Bell, J., Springer Semin Immunopathol 14 (1992) 59-78). In particular, the class II MHC alleles *HLA-DRB1*0101*, **0401*, **0404* and **0405* in several ethnic groups

increase the susceptibility to RA (Reveille, J., Curr Opin Rheumatol 10 (1998) 187-200). E.g. more than 90% of RF-positive RA patients carry one of these susceptibility alleles. HLA class II molecules are MHC-surface proteins that bind antigenic peptides within the cell and present them on the surface of antigen-presenting cells for interaction with the T cell receptors of $CD4^+$ helper T lymphocytes, thereby initiating a cellular immune response (Banchereau, J. & Steinman, R.M., Nature 392 (1998) 245-252). The RA-association of particular HLA class II molecules together with the presence of large numbers of activated $CD4^+$ T cells in synovial tissue has supported the model of disease induction in which disease-associated HLA-DR molecules present disease-relevant (e.g. synovial) autoantigens and cause stimulation and expansion of synovial T cells, which then drive the inflammatory process (Striebich, C.C. et al., J Immunol 161 (1998) 4428-4436).

MHC class II HLA-DR (short: DR) proteins are heterodimers consisting of monomorphic α- and extremely polymorphic β-chains that bind peptide antigens in a peptide binding groove. This groove generally has four major pockets to accept side chains at relative positions 1, 4, 6 and 9 of the peptide (Stern, L.J. et al., Nature 368 (1994) 215-221). The allelic variations between HLA class II molecules account for the differential ability to bind antigenic peptides. This is the rationale why individuals differing in their HLA alleles have divergent antigenic peptide repertoirs, thereby leading to differences in the quality of immune responses (Messaoudi, I. et al., Science 298 (2002) 1797-1800).

Peptides bound by class II MHC molecules are typically longer and more heterogeneous in size (11-25 amino acids) than the peptides bound by class I MHC molecules (8-10 amino acids). This difference arises because the peptide binding groove of class II proteins is open and while peptides are gripped in the midle, their ends can extend out of the groove in a variable fashion (Jones, E.Y., Curr Opin Immunol 9 (1997) 75-79). As a consequence, class II molecules typically bind sets of overlapping peptides that share a common core sequence, termed "T cell epitope", but have different lengths.

More than a decade ago, it was recognized that the DRβ chains encoded by RA-linked DRB1 alleles, although exhibiting polymorphic differences, all share a stretch of identical or almost identical amino acids at positions 67-74, known as the "shared epitope" (Gregersen, P.K. et al., Arthritis Rheum 30 (1987) 1205-1213). Since immunity to autoantigens has been regarded central to the pathogenesis of RA, it was hypothesized that the shared epitope could impose disease linkage on the respective DR molecules by at least two different mechanisms: first, by selecting the relevant autoantigenic peptides for presentation, and second, by selecting the appropriate autoreactive T cell specificities during ontogeny. The three-dimensional structure of DR molecules has indeed revealed that the shared epitope is located in the center of the α-helix flanking one side of the

peptide binding groove (Stern, L.J. et al., Nature 368 (1994) 215-221). Thus, strategically this shared epitope region is positioned in such a way that it can interact with both bound peptide and T cell receptor.

However, one of the unresolved mysteries in rheumatology research is the question what are the key arthritogenic antigens and epitopes in man that trigger the onset and the development of RA. Although autoantibodies of different specificity have been identified in serum and synovial fluid of patients it is often unclear whether the antigens which were released at the time of cartilage degradation, were initiating pathogenicity or whether they are merely a consequence of antigen spreading as a result of inflammation (Corrigall, V.M. & Panayi G.S., Crit Rev Immunol 22 (2002) 281-293). Furthermore it is difficult to define pathogenic mechanisms in which the antigen is present throughout the body, including the joint, but the pathology is targeted solely or predominately to the joint.

Autoantigens

The large number of possible autoantigens in RA is derived from studies using sera or, less frequently, T cells from patients with established chronic RA. One of the most convincing joint-specific antigen that has been proposed in the context of DR molecules, is type II collagen (CII), the predominant protein in articular cartilage. Autoantibodies against CII were found in elevated concentrations in the serum and joints of RA patients although it is not yet clear whether anti-CII antibodies are pathogenic in RA (Banerjee, S. et al., Clin Exp Rheumatol 6 (373-380). Snowden and coworkers have shown that peripheral blood T cells from RA patients proliferated to CII, most pronounced in those patients with anti-CII antibodies. However, the response was seen only in 50% of patients (Snowden, N. et al., Rheumatology 40 (1997) 1210-1218). In a mouse model immunization with CII was shown to induce arthritis in mice expressing the class II MHC alleles *DRB1*0401* and **0101* (Rosloniec, E.F. et al., J Exp Med 185 (1997) 1113-1122; Rosloniec, E.F. et al., J immunol 160 (1998) 2573-2578). The immunodominant epitope in both **0401* and **0101* transgenic mice was localized to peptides within residues 261-273 of human CII (Fugger, L. et al., Eur J Immunol 26 (1996) 928-933). The same epitope of CII was capable of stimulating a T cell response in RA patients, particularly in the early stages of disease. Synovial fluid T cells were especially responsive (Kim, H.Y. et al., Arthritis Rheum 42 (1999) 2085-2093).

Although other cartilage proteins have been proposed as RA candidate antigens, DR4-binding epitopes have been defined only for human cartilage glycoprotein 39 (HCgp39). This protein is secreted by synovial cells and articular chondrocytes and its expression is upregulated in plasma and joints during inflammation (Vos, K. et al., Ann Rheum Dis 59 (2000) 544-548). Similar to CII, HCgp39 treatment induces arthritis in

mice. In addition a HCgp39 response of peripheral blood T cells from RA patients was detected (Verheijden, G.F. et al., Arthritis Rheum 40 (1997) 1115-1125). The predominant epitope recognized by T cells in DR4 patients was defined between residues 263-275 and identical to the immunodominant epitope found in *DRB1*0401*-transgenic mice after immunization with native HCgp39 (Cope, A.P. et al., Arthritis Rheum 42 (1999) 1497-1507). Although not disease specific, responses to this peptide did correlate with disease activity in RA patients (Baeten, D. et al., Arthritis Rheum 43 (2000) 1233-1243). Antibodies to HCgp39, however, have also been detected in the sera of patients with inflammatory diseases, such as inflammatory bowel disease and systemic lupus erythematosus (SLE), albeit at a lower level than in RA.

In an attempt to track antigen-specific T cells in RA, soluble peptide-DR4 tetrameric complexes were used to detect synovial $CD4^+$ T cells reactive with CII or HCgp39 in $DR4^+$ patients (Kotzin, B.L. et al., PNAS 97 (2000) 291-296). The CII-DR4 complex bound in a specific manner to CII peptide-reactive T cell hybridomas, but did not stain a detectable fraction of synovial $CD4^+$ cells. Almost similar results were obtained with the HCgp39-DR4 complex suggesting that the major oligoclonal $CD4^+$ T cell expansions present in RA joints are not specific for the dominant CII and HCgp39 determinants described above.

In summary, despite some strong indications for a CII and HCgp39 association with RA, the evidence that they are important antigens in RA is scanty. A direct proof that peptides of CII or HCgp39 are presented in a class II MHC-restricted manner by antigen-presenting cells with subsequent stimulation and activation of synovial $CD4^+$ T cells is still lacking. Furthermore a major problem of animal models is their unknown relevance to RA as CII-induced arthritis by immunizing rats or mice differs in many respects from RA.

Naturally processed MHC class II-associated peptides

An alternative strategy to the identification of RA-specific autoantibodies and T cells relies on the sequence analysis of naturally processed peptide antigens bound to MHC class II molecules. With the help of monoclonal antibodies, class II MHC molecules conferring susceptibility to RA can be purified from cognate cells. RA-associated peptide antigens can be acid-eluted from purified HLA class II molecules. The mixture of small peptides can be separated by HPLC and the peptide sequence be determined by Edman sequencing or mass spectrometry. Due to limitations with peptide purification and sequencing techniques, peptide sequences were, as yet, only obtained from MHC molecules that have been isolated from cultured B cell lines or large amounts of tissue, and the analysis was restricted to a few abundant peptides (Kropshofer et al., J.Exp.Med. 175 (1992) 1799-1803; Chicz, R.M. et al., J Exp Med 178 (1993) 27-47). As a result of the development of high-resolution microcapillary HPLC columns and more sensitive mass spectrometers, MHC-bound

peptides can be analyzed more efficiently (Dongre, A.R. et al., Eur J Immunol 31 (2001) 1485-1494; Engelhard, V.H. et al., Mol Immunol 39 (2002) 127-137).

In the present invention a modified peptide isolation and sequencing technique was used to investigate the peptide antigen repertoire of HLA-DR4 molecules derived from autologous dendritic cells (DCs) which were pulsed with serum or synovial fluid derived from RA patients. The main advantage of this innovative approach is the usage of human DCs that are professionals in RA-relevant antigen processing and presentation, instead of using transgenic animal models or artificial B cell lines.

DCs are enriched in rheumatoid synovial fluid and tissue and are derived from circulating immature precursors (Thomas, R. et al., J Immunol 152 (1994) 2613-2623). They are the most potent antigen-presenting cells which express high levels of MHC molecules together with a variety of accessory molecules (Mellman, I. et al., Trends Cell Biol 8 (1998) 231-237). In a most recent study, it was shown that *ex vivo* differentiated human DCs and macrophages that are phenotypically similar to antigen-presenting cells from RA synovial joints, were capable of generating and presenting immunodominant epitopes from CII and HCgp39 (Tsark, E.C. et al., J Immunol 169 (2002) 6625-6633). DC have the capacity to prime $CD4^+$ helper T cells and to effectively activate cytotoxic $CD8^+$ T cells (Ridge, T. et al., Nature 393 (1998) 474-478). Thus, peptides bound to MHC class II molecules and presented by DCs play a superior role in the pathogenesis of diseases involving T cell-driven immune responses.

Therefore, the problem posed by the lack of knowledge of MHC class II restricted antigenic peptides for RA is solved by providing novel naturally-processed MHC class II associated RA antigenic peptides and the polypeptides they are derived from as markers for RA.

The present invention provides novel naturally-processed antigenic peptides which are candidate RA markers in erosive and non-erosive RA. These antigenic peptides are presented by human MHC class II HLA-DR molecules derived from dendritic cells which were pulsed with serum or synovial fluid derived from patients with established erosive or non-erosive RA. The MHC class II antigenic peptide of the invention are comprising (a) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57, or (b) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57 with additional N-and C-terminal flanking sequences of a corresponding sequence selected from the group consisting of SEQ ID NOs. 1 to 39, and originate from interferon-γ-inducible lysosomal

thiol reductase, apolipoprotein B-100, inter- α-trypsin inhibitor heavy chain H4, complement C4, complement C3, SH3 domain-binding glutamic acid-rich-like protein 3, interleukin-4-induced protein 1, hemopexin, and Hsc70-interacting protein. The present invention also provides these antigenic peptides and the proteins they are derived from as markers for erosive and/or non-erosive RA. Moreover, these antigenic peptides linked to MHC class II molecules, as well as antibodies reactive with said antigenic peptides, nucleic acids encoding said antigenic peptides, and nucleic acid constructs, host cells and methods for expressing said antigenic peptides are provided. Further methods are provided for isolating and identifying RA antigenic peptides.

Fig. 1: Diagram of Dendritic cell (DC)-mediated analysis of tissue samples: Dendritic cells (DCs), the most specialized antigen-presenting cells (APCs), are brought in contact with an antigen source (e.g. synovial fluid) under optimal conditions for antigen uptake and antigen processing. As a control, DCs are cultured under the same conditions in the absence of synovial fluid antigens. After maturation of DCs, antigen-loaded MHC class II molecules are purified and the respective MHC class II-associated antigenic peptides are isolated and identified.

Fig. 2A: ION-TRAP MS Base Peak Chromatogram of MHC class II-associated antigenic peptides that were isolated from dendritic cells pulsed with the serum of a RA patient. The peptides were eluted directly from a RP-C18-HPLC column into the ion trap mass spectrometer for immediate MS/MS identification. The numbers indicate the retention times (upper value) and the molecular masses (lower value) of the most prominent peptide peaks in the mixture at the respective time.

Fig. 2B: ION-TRAP MS spectrum of antigenic peptides at a retention time of 65.4 min. The marked peak was further fragmented and corresponded to a doubly charged peptide ion from the inter-alpha-trypsin inhibitor ITIH4 (cf. table 3).

Fig. 2C: ION-TRAP MS/MS spectrum of the doubly charged peptide ion at m/z 977.1. The fragmentation masses, together with the mass of the parent ion, were searched against a non-redundant human database by using the SEQUEST algorithm. The retrieved sequence MPKNVVFVIDKSGSMSGR (one-letter-code) corresponded to the dominant epitope ITIH4 (271-288) of the inter-alpha-trypsin inhibitor. The positions of the assigned series of N-terminal B-ions and C-terminal Y-ions are marked.

The antigenic peptides of the invention are peptides, which are associated with and presented by MHC molecules and thereby can have the potential to activate or tolerize T cells. Antigenic peptides presented by MHC class II molecules are therefore MHC class II associated or MHC class II antigenic peptides, whereas antigenic peptides presented by MHC class I molecules are MHC class I associated or MHC class I antigenic peptides.

Peptides which are derived from proteins that are encoded in the genome of the body or an APC are denoted as "self-peptides". The main function of self-peptides presented by DCs in the peripheral lymphoid organs is thought to be the induction of T cell tolerance to self-proteins. Tolerance is the failure to respond to an antigen; when that antigen is borne by self tissues, tolerance is called self tolerance.

Antigens which are derived from an individual's own body are called "self antigens" or "autoantigens". An adaptive immune response directed against self antigens is called an autoimmune response. Likewise, adaptive immunity specific for self antigens is called autoimmunity. Autoreactivity describes immune responses directed against self antigens. RA is probably due to an autoimmune response that is based on the involvement of autoreactive T cells and/ or autoreactive antibodies. Immunogenic peptide includes, but is not limited to, an antigenic peptide capable of causing or stimulating a cellular or humoral immune response. Such peptides may also be reactive with antibodies.

Peptides derived from proteins encoded in the genome of bacteria, viruses or other foreign invaders and which differ from self-proteins are called "foreign antigenic" or "foreign" peptides. They are able to elicit a T cell response against foreign proteins they are derived from.

RA antigenic peptides are self-peptides that function as self antigens and as a consequence of the disease erroneously trigger autoreactivity against self tissues.

The present invention provides a MHC class II antigenic peptide comprising (a) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57, or (b) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57 with additional N-and C-terminal flanking sequences of a corresponding sequence selected from the group consisting of SEQ ID NOs. 1 to 39. Preferably, the MHC class II antigenic peptide has a length of less than 26 amino acids, more preferably a length of 11 to 25 amino acids. Even more preferred is the antigenic peptide of the invention with a length of 11 to 19 amino

acids. Most preferred is the antigenic peptide of the invention consisting of the peptide binding motif comprising the four anchor amino acids.

The present invention also provides a MHC class II antigenic peptide comprising (a) at least the amino acid sequence of the peptide binding motif of SEQ ID NO. 49, or (b) at least the amino acid sequence of the peptide binding motif of SEQ ID NO. 49 with additional N-and C-terminal flanking sequences of a corresponding sequence selected from the group consisting of SEQ ID NOs. 1 to 3.

Furthermore, a MHC class II antigenic peptide is provided comprising (a) at least the amino acid sequence of the peptide binding motif of SEQ ID NO. 50, or (b) at least the amino acid sequence of the peptide binding motif of SEQ ID NO. 50 with additional N- and C-terminal flanking sequences of the corresponding sequence of SEQ ID NO. 5.

The MHC class II associated novel antigenic peptides of the invention originate from interferon-γ-inducible lysosomal thiol reductase (SEQ ID NOs. 1 to 3), apolipoprotein B-100 (SEQ ID NOs. 4 and 5), inter-α-trypsin inhibitor heavy chain H4 (SEQ ID NOs. 6 to 12), complement C4 (SEQ ID NOs. 13 to 18), complement C3 (SEQ ID NOs. 19 to 23), SH3 domain-binding glutamic acid-rich-like protein 3 (SEQ ID NOs. 24 to 27), interleukin-4-induced protein 1 (SEQ ID NOs. 28 to 30), hemopexin (SEQ ID NOs. 31 to 35), and Hsc70-interacting protein (SEQ ID NOs. 36 to 39).

The single peptide binding groove of MHC class II molecules is about 25 Å long, but in contrast to MHC class I molecules, both sides are open (Stern LJ et al., Nature 1994; 368, 215-221). Thus, naturally processed antigenic peptides eluted from human MHC class II molecules have a minimal length of about 11 residues and attain a maximal length of about 25 residues (Chicz RM et al., J Exp Med 1993; 178, 27-47).

The stability of the MHC-peptide interaction is determined by more than a dozen hydrogen bonds involving the peptide backbone and the complementarity between specificity pockets of the binding groove and appropriately located amino acid side-chains of the peptide. The amino acids of the peptide fitting into the respective pockets were named "anchor" residues. With regard to most HLA-DR alleles, these anchors are located at relative positions P1, P4, P6 and P9. The combination of amino acids at these 4 anchor positions conferring high-stability binding to the respective HLA-DR allelic product and vary from allele to allele. The peptide binding motif is defined herein as the sequence of nine amino acids comprising the four anchor amino acids. The peptide binding motif of the MHC class II antigenic peptide of the invention is depicted in SEQ ID NO. 49 for the peptides derived from interferon-γ-inducible lysosomal thiol reductase (SEQ ID NOs. 1 to 3), in SEQ ID NO. 50 for the peptides derived from apolipoprotein B-100 (SEQ ID NOs. 4

and 5), in SEQ ID NO. 51 for the peptides derived from inter-α-trypsin inhibitor heavy chain H4 (SEQ ID NOs. 6 to 12), in SEQ ID NO. 52 for the peptides derived from complement C4 (SEQ ID NOs. 13 to 18), in SEQ ID NO. 53 for the peptides derived from complement C3 (SEQ ID NOs. 19 to 23), in SEQ ID NO. 54 for the peptides derived from SH3 domain-binding glutamic acid-rich-like protein 3 (SEQ ID NOs. 24 to 27), in SEQ ID NO. 55 for the peptides derived from interleukin-4-induced protein 1 (SEQ ID NOs. 28 to 30), in SEQ ID NO. 56 for the peptides derived from hemopexin (SEQ ID NOs. 31 to 35), and in SEQ ID NO. 57 for the peptides derived from Hsc70-interacting protein (SEQ ID NOs. 36 to 39). The peptide binding motif may also comprise at least one, at least two, at least three, at least four or at least five modifications of the amino acid sequence while still attaining the binding capacity of the non-modified peptide binding motif. Preferably, the modified peptide binding motif comprises at least three of the four anchor amino acids of the non-modified peptide binding motif. The amino acid modification may be a conservative amino acid substitution as described below.

Additional binding energy is provided by hydrogen bonds involving residues in front of the P1 anchor and behind the P9 anchor. In agreement with that, in most naturally processed peptides the nonameric core-region (P1-P9) is N- and C-terminally flanked by 3-4 residues. Hence, the majority of peptides are 15-17-mers. Longer peptides protrude from the groove, thereby allowing access of exopeptidases which are trimming both ends.

Therefore, the MHC class II antigenic peptide of the invention comprising (a) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57, or (b) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57 with additional N- and C-terminal flanking sequences of a corresponding sequence selected from the group consisting of SEQ ID NOs. 1 to 39, preferably comprises additional N- and C-terminal flanking amino acid residues providing additional binding energy.

Preferably, the MHC class II antigenic peptide of the present invention has a binding capacity to the corresponding MHC class II molecule of between one tenth and ten-fold the IC_{50} of a corresponding peptide selected from the group consisting of SEQ ID NOs. 1 to 39. The binding capacity of a peptide is measured by determining the concentration necessary to reduce binding of a labelled reporter peptide by 50%. This value is called IC_{50}. A MHC class II antigenic peptide of the invention maintains its binding capacity to the relevant HLA class II molecules as long as it attains IC_{50} values between one tenth and 10-fold the IC_{50} of the established reference peptides.

Since peptide trimming occurs in an individual fashion both before and after binding into the peptide binding groove, the occurrence of several truncation variants sharing a common nonameric core region is a common feature of MHC class II-bound peptides. Importantly, it was shown that C- or N-terminal truncation variants of the same epitope can trigger divergent T cell responses (Arnold et al., (2002) J. Immunol. 169, 739-749).

Several parameters can be envisaged that have an influence on the relative abundance of truncation variants of a particular epitope, e.g. the abundance and integrity of the antigen of relevance, antigen-associated proteins, the abundance of proteases, the type of proteases available and the supply with competitive antigens and/or peptides. Since the antigen supply is a major characteristic that may correlate with the origin of a sample, the ratio of particular truncation variants of an epitope can be of diagnostic value.

A peptide of the invention is a peptide which either has no naturally-occurring counterpart (e.g., such as an mutated peptide antigen), or has been isolated, i.e., separated or purified from components which naturally accompany it, e.g., in tissues such as pancreas, liver, spleen, ovary, testis, muscle, joint tissue, neural tissue, gastrointestinal tissue, or body fluids such as blood, serum, synovial fluid or urine. Typically, the peptide is considered "isolated" when a preparation comprising a peptide of the invention consists to at least 70%, by dry weight of said peptide and to less than 30% of the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, a preparation of a peptide of the invention consists of at least 80%, more preferably at least 90%, and most preferably at least 99%, by dry weight, the peptide of the invention. Since a peptide that is chemically synthesized is, by its nature, separated from the components that naturally accompany it, the synthetic peptide is "isolated".

The invention further provides analogs of the antigenic peptide of the invention. The term analog includes any peptide which displays the functional aspects of these antigenic peptides comprising the binding capacity IC_{50} and the recognition by antibodies and cells of the immune system. Analogs exhibit essentially the same IC_{50} as the corresponding reference peptide. The term analog also includes conservative substitutions or chemical derivatives of the peptides.

The term "analog" includes any polypeptide having an amino acid residue sequence substantially identical to the sequences described herein in which one or more residues

have been conservatively substituted with a functionally similar residue and which displays the functional aspects of the peptides as described herein. Examples of conservative substitutions include the substitution of one non-polar (hydrophobic) residue such as phenylalanine, tyrosine, isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between threonine and serine, the substitution of one basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another.

The phrase "conservative substitution" also includes the use of a chemically derivatized amino acid in place of a non-derivatized amino acid. "Chemical derivative" refers to a subject polypeptide having one or more amino acids chemically derivatized by reaction of a functional side group. Examples of such derivatized molecules include for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups, acetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Also included as chemical derivatives are those proteins or peptides, which contain one or more naturally-occurring amino acid derivative of the twenty standard amino acids. For examples: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine or citrulline may be substituted for lysine.

The MHC class II antigenic peptides of the invention and the proteins they are derived from can be used as markers in diagnosis of RA and in therapy as anti-RA vaccines. The term marker as used herein refers to a biomolecule, preferably a peptide or a polypeptide, which is expressed in a group of patients with a diagnosed disease, e.g. RA, and attains an abundance that is significantly increased or decreased as compared to a control group.

The marker of the present invention may be used as a prognostic marker to predict the susceptibility to a disease, e.g., to predict the susceptibility to RA, as a diagnostic marker for the diagnosis of a disease, e.g. for the diagnosis of RA, as a differential diagnostic marker to differentiate between different forms of a disease, e.g., to differentiate between different forms of RA, as a prognostic marker for the prediction of the outcome of

a disease, e.g., for the prognosis of RA, and as a response marker to determine the efficacy of a therapeutic regime, e.g., as a response marker in the treatment of RA.

In a further embodiment, the MHC class II antigenic peptide comprising (a) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57, or (b) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57 with additional N-and C-terminal flanking sequences of a corresponding sequence selected from the group consisting of SEQ ID NOs. 1 to 39 is used as a marker for erosive and/or non-erosive RA.

In a further embodiment, the MHC class II antigenic peptide comprising (a) at least the amino acid sequence of the peptide binding motif of SEQ ID NO. 49, or (b) at least the amino acid sequence of the peptide binding motif of SEQ ID NO. 49 with additional N- and C-terminal flanking sequences of a corresponding sequence selected from the group consisting of SEQ ID NOs. 1 to 3 is used as a marker for non-erosive RA.

In a further embodiment, the MHC class II antigenic peptide comprising (a) at least the amino acid sequence of the peptide binding motif of SEQ ID NO. 50, or (b) at least the amino acid sequence of the peptide binding motif of SEQ ID NO. 50 with additional N- and C-terminal flanking sequences of the corresponding sequence of SEQ ID NO. 5 is used as a marker for erosive RA.

In a further embodiment, the MHC class II antigenic peptides of the invention as described above are provided linked to a MHC class II molecule.

Multimers (e.g., dimers, trimers, tetramers, pentamers, hexamers or oligomers) of a class II MHC molecule containing a covalently or non-covalently bound peptide according to the present invention, if conjugated with a detectable label (e.g., a fluorescent moiety, a radionuclide, or an enzyme that catalyzes a reaction resulting in a product that absorbs or emits light of a defined wavelength) can be used to quantify T cells from a subject (e.g., a human patient) bearing cell surface receptors that are specific for, and therefore will bind, such complexes. Relatively high numbers of such T cells are likely to be diagnostic of disease or an indication that the T cells are involved in immunity to the disease. In addition, continuous monitoring of the relative numbers of multimer-binding T cells can be useful in establishing the course of a disease or the efficacy of therapy. Such assays have been developed using tetramers of class I MHC molecules containing an HIV-1-derived or an influenza virus-15 derived peptide (Altman et al. (1996), Science 274:94-96; Ogg et al.

(1998), Science 279:2103- 21061), and corresponding class II MHC multimers would be expected to be similarly useful. Such complexes could be produced by chemical cross-linking of purified class II MHC molecules assembled in the presence of a peptide of interest or by modification of already established recombinant techniques for the production of class II MHC molecules containing a single defined peptide (Kazono et al. (1994), Nature 369:151-154; Gauthier et al. (1998), Proc. Natl. Acad. Sci. U.S.A. 95:11828-118331). The class II MHC molecule monomers of such multimers can be native molecules composed of full-length alpha and beta chains. Alternatively, they can be molecules containing either the extracellular domains of the alpha and beta chains or the alpha and beta chain domains that form the "walls" and "floor" of the peptide-binding cleft.

The invention also relates to an antibody, fragments or derivatives thereof, directed to and reactive with the above-described MHC class II antigenic peptides. The general methodology for producing antibodies is well known and is disclosed per example in Kohler and Milstein, 1975, Nature 256,494 or in J. G. R. Hurrel, Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press Inc., Boco Raron, FL (1982). The antibodies can be polyclonal or, preferably, monoclonal, or antibody fragments like be F (ab') 2, Fab, Fv or scFv. The antibodies of the present invention may also be humanized (Merluzzi S. et al., (2000), Adv. Clin. Path., 4(2): 77-85) or human antibodies (Aujame L. et al., Hum. Antibodies, (1997), 8(4): 155-168).

The present invention also provides a nucleic acid molecule encoding a MHC class II antigenic peptide of the invention comprising (a) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57, or (b) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57 with additional N- and C-terminal flanking sequences of a corresponding sequence selected from the group consisting of SEQ ID NOs. 1 to 39. Preferably, the nucleic acid molecule is a DNA molecule.

Furthermore, a nucleic acid molecule is provided encoding a MHC class II antigenic peptide of the invention linked to a MHC class II molecule.

This invention also provides a recombinant nucleic acid construct comprising the nucleic acid molecules as described above, operably linked to an expression vector. Expression vectors suitable for use in the present invention comprise at least one expression control element operably linked to the nucleic acid sequence encoding the antigenic peptide or the antigenic peptide linked to a MHC class II molecule. The recombinant expression construct may be a DNA construct.

The expression control elements are inserted in the vector to control and regulate the expression of the nucleic acid sequence encoding the antigenic peptide of the invention. Examples of expression control elements include, but are not limited to, lac system, operator and promoter regions of phage lambda, yeast promoters and promoters derived from polyoma, adenovirus, retrovirus or SV40. Additional preferred or required operational elements include, but are not limited to, leader sequence, termination codons, polyadenylation signals and any other sequences necessary or preferred for the appropriate transcription and subsequent translation of the nucleic acid sequence in the host system. It will be understood by one skilled in the art that the correct combination of required or preferred expression control elements will depend on the host system chosen. It will further be understood that the expression vector should contain additional elements necessary for the transfer and subsequent replication of the expression vector containing the nucleic acid sequence in the host system. Examples of such elements include, but are not limited to, origins of replication and selectable markers. It will further be understood by one skilled in the art that such vectors are easily constructed using conventional methods (www.cellbio.com/protocols.html) or are commercially available.

Another aspect of this invention relates to a host organism or a host cell into which a recombinant nucleic acid construct comprising the nucleic acid molecules as described above, operably linked to an expression vector, has been inserted. The host cells transformed with the nucleic acid constructs encompassed by this invention include eukaryotes, such as animal, plant, insect and yeast cells and prokaryotes, such as E. coli. The means by which the nucleic acid construct carrying the nucleic acid sequence may be introduced into the cell include, but are not limited to, microinjection, electroporation, transduction, or transfection using DEAE-dextran, lipofection, calcium phosphate or other procedures known to one skilled in the art (Sambrook et al. (1989) in "Molecular Cloning. A Laboratory Manual", Cold Spring Harbor Press, Plainview, New York).

In a preferred embodiment, eukaryotic expression vectors that function in eukaryotic cells are used. Examples of such vectors include, but are not limited to, retroviral vectors, vaccinia virus vectors, adenovirus vectors, herpes virus vector, fowl pox virus vector, plasmids, or the baculovirus transfer vectors. Preferred eukaryotic cell lines include, but are not limited to, COS cells, CHO cells, HeLa cells, NIH/3T3 cells, 293 cells (ATCC# CRL15731), T2 cells, dendritic cells, monocytes or Epstein-15 Barr Virus transformed B cells.

An antigenic peptide of the invention can be obtained, for example, by extraction from a natural source (e.g., elution from MHC II molecules); by expression of a recombinant nucleic acid encoding the peptide; or by chemical synthesis. A peptide that is

produced in a cellular system different from the source from which it naturally originates is "isolated," because it will be separated from components which naturally accompany it. The recombinant peptide expressed by a host organism can be obtained as a crude lysate or can be purified by standard protein purification procedures known in the art which may include differential precipitation, size exclusion chromatography, ion-exchange chromatography, isoelectric focusing, gel electrophoresis, affinity, and immunoaffinity chromatography and the like. The extent of isolation or purity can be measured by any appropriate method, e.g. mass spectrometry or HPLC analysis. The peptides may be prepared synthetically by procedures described in Merrifield, (1986) Science 232: 341-347, and Barany andMerrifield, The Peptides, Gross and Meienhofer, eds (N. Y., Academic Press), pp. 1-284 (1979). The synthesis can be carried out in solution or in solid phase or with an automatized synthesizer (Stewart and Young, Solid Phase Peptide Synthesis, 2nd ed., Rockford Ill., Pierce Chemical Co. (1984)).

Therefore, the present invention further provides a method for producing a MHC class II antigenic peptide comprising (a) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57, or (b) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57 with additional N-and C-terminal flanking sequences of a corresponding sequence selected from the group consisting of SEQ ID NOs. 1 to 39, comprising the steps of culturing the host cell containing a recombinant nucleic acid construct as described above under conditions allowing expression of said peptide and recovering the peptide from the cells or the culture medium.

In a further embodiment of the present invention, a method is provided for isolating and identifying MHC class II associated RA antigenic peptides in femtomolar amounts, which method comprises (a) providing immature dendritic cells in a number comprising 0.1 to 5 μg MHC class II molecules; (b) contacting the cells of (a) with serum or synovial fluid and inducing maturation of dendritic cells by adding TNFalpha; (c) isolating class II MHC molecule-antigenic peptide complexes from the cells with methods comprising solubilization of the cells and sequestration of the complexes of MHC class II molecules with antigenic peptides by immunoprecipitation or immunoaffinity chromatography; (d) washing the sequestered complexes of MHC class II molecules with antigenic peptides with water in an ultrafiltration tube; (e) eluting the associated antigenic peptides from the MHC class II molecules at 37°C with diluted trifluoro acetic acid, and (f) separating, detecting and identifying the isolated peptides by liquid chromatography and mass spectrometry. Furthermore, in step (f) of the method, the liquid chromatography

comprises a first linear elution step from the reversed-phase material with a volume sufficient to elute the majority of contaminants prior to peptide elution. Moreover, the method may further comprise (g) analyzing the identified peptides by methods comprising a database and a software developed to perform comparative data analysis across multiple datasets.

The amount of tissue or bodily fluid necessary to obtain e.g. 100 ng MHC class II molecules depends on the number of cells that do express MHC class II and on the expression rate of MHC class II molecules: e.g. 100 ng of MHC class II are equivalent to about 2×10^5 mature DCs or 5 to 10×10^6 peripheral blood monocytes or about 5×10^7 peripheral blood mononuclear cells which can be obtained from about 50 ml of blood.

For the purification of class II MHC molecule-antigenic peptide complexes from cells or tissue, the membranes of the cells or tissue have to be solubilized. Cell lysis may be carried out with methods known in the art, e.g. freeze-and-thaw cycles and the use of detergents, and combinations thereof. Preferred lysis methods are solubilization using detergents, preferably TX-100, NP40, n-octylglucoside, Zwittergent, Lubrol, CHAPS, most preferably TX-100 or Zwittergent 3-12. Cell debris and nuclei have to be removed from cell lysates containing the solubilized receptor-peptide complexes by centrifugation. Therefore, the complexes of class II MHC molecules with antigenic peptides are isolated from the cells with methods comprising solubilization with a detergent.

Furthermore, the MHC class II molecule-peptide complexes are purified from cell lysates by methods comprising immunoprecipitation or immunoaffinity chromatography. For the immunoprecipitation or immunoaffinity chromatography, antibodies specific for MHC class II molecules and suitable for these methods are used. The specific antibodies are preferably monoclonal antibodies, and are covalently or non-covalently e.g. via Protein A, coupled to beads, e.g. sepharose or agarose beads. A selection of the broad panel of anti-HLA antibodies used in the prior art comprises: anti-HLA-DR antibodies: L243, TU36, DA6.147, preferably L243; anti-HLA-DQ antibodies: SPVL3, TU22, TU169, preferably TU22 and TU169; anti-HLA-DP antibody B7/21 and anti-HLA-A,B,C antibodies W6/32 and B9.12.

Monoclonal antibodies specific for different MHC class II molecules may be commercially obtained (e.g. Pharmingen, Dianova) or purified from the supernatant of the respective hybridoma cells using Protein A- or Protein G- affinity chromatography. Purified monoclonal antibodies may be coupled by various methods known in the art, preferably by covalently coupling antibody amino groups to CNBr-activated sepharose.

Immunoisolation of MHC molecules may be performed by incubating the antibody-beads with the cell lysate under rotation for several hours or chromatographically by pumping the cell lysate through a micro-column. Washing of the antibody-beads may be performed in eppendorf tubes or in the microcolumn. The efficacy of the immunoprecipitation may be analysed by SDS-PAGE and western blotting using antibodies recognizing denatured MHC molecules (anti-HLA-DRalpha: 1B5; anti-HLA class I: HC10 or HCA2).

The sequestered MHC class II molecule-peptide complexes are washed with water or low-salt buffer before elution in order to remove residual detergent contaminants. The low salt buffer may be a Tris, phosphate or acetate buffer in a concentration range of 0.5 – 10 mM, preferably in a concentration of 0.5 mM. In a more preferred embodiment, the MHC class II molecule -peptide complexes are washed with ultrapure water (sequencing grade) conventionally used for HPLC analysis, preferably with ultrapure (sequencing grade) water from MERCK. The washing step may be carried out by ultrafiltration. The ultrafiltration may be carried out in an ultrafiltration tube with a cut-off of 30 kD, 20 kD, 10 kD or 5 kD, preferably of 30 kD and a tube volume of 0.5 – 1.0 ml ("Ultrafree" tubes; Millipore). The washing in the ultrafiltration tube may be carried out 4 to 12 times, preferably 6 to 10 times, with a volume of 10 to 20 times the volume of the beads carrying the receptor-peptide complexes, preferably with a volume of 15 times the beads. The eluted peptides may be separated from the remaining MHC class II molecules using the same ultrafiltration tube. The eluted peptides may then be lyophilized.

By eluting the peptides from the MHC class II molecules, a complex mixture of naturally processed peptides derived from the source of potential antigen and from polypeptides of intra- or extracellular origin, is obtained. Only after elution, peptides can be separated and subjected to sequence analysis.

The antigenic peptides in the method of the present invention may be eluted by a variety of methods known in the art, preferably by using diluted acid, e.g., diluted acetonitrile (Jardetzky TS et al., Nature 1991 353, 326-329), diluted acetic acid and heating (Rudensky AY et al., Nature 1991, 353, 622-626; Chicz RM et al., Nature 1992, 358, 764-768) or diluted trifluoro acetic acid at 37°C (Kropshofer H et al., J Exp Med 1992, 175, 1799-1803). Most preferably, the peptides are eluted at 37°C with diluted trifluoro acetic acid.

The isolated antigenic peptides are then separated, detected and identified. By detecting it is understood that the amino acid sequence of the individual peptides in the mixture of isolated antigenic peptides is elucidated by methods adequate to detect and sequence femtomolar amounts of peptides. By identifying it is understood that it is

established from which proteins or polypeptides the antigenic peptides are derived and which sequence they constitute within these proteins or polypeptides.

In a first step, the complex mixture of eluted peptides may be separated by one of a variety of possible chromatographic methods, e.g. by reversed phase, anion exchange, cation exchange chromatography or a combination thereof. Preferably, the separation is performed by C18-reverse phase chromatography or by reversed-phase / cation exchange two-dimensional HPLC, denoted as MudPit (Washburn MP et al., Nat Biotechnol., (2001), 19, 242-247).

The separation is done in a HPLC mode utilizing fused-silica micro-capillary columns which are either connected to a nano-flow electrospray source of a mass spectrometer or to a micro-fractionation device which spots the fractions onto a plate for MALDI analysis.

Liquid chromatography comprises peptide fractionation by the use of a strong ion exchange material and a hydrophobic reversed-phase material. For the elution of the peptides from the ion exchange and reversed-phase material different elution programs are run one after another comprising elutions with salt and with organic solvents, e.g., acetonitrile. The elution from the reversed-phase material is conducted in several steps of linear gradients of different lengths and slopes. A contamination in the sample to be fractionated may be any contamination whose elution competes with the detection of the peptide peaks in the mass spectrometer. Therefore, in order to prevent simultaneous elution, the contaminants have to be eluted with a sufficient solvent volume prior to the peptide elution step. Depending on the column used for liquid chromatography the solvent volume sufficient to elute the contaminants prior to the peptide elution step may be 100 to 200 times the column volume.

A variety of mass spectrometric techniques are suitable, preferably MALDI-post source decay (PSD) MS or electrospray ionization tandem mass spectrometry (ESI-MS), most preferably ion-trap ESI-MS.

The sequences of the individual peptides can be determined by means known in the art. Preferably, sequence analysis is performed by fragmentation of the peptides and computer-assisted interpretation of the fragment spectra using algorithms, e.g. MASCOT or SEQUEST. Both computer algorithms use protein and nucleotide sequence databases to perform cross-correlation analyses of experimental and theoretically generated tandem mass spectra. This allows automated high through-put sequence analysis.

The isolated and identified antigenic peptides of the invention can be validated by the MHC binding motif, the MHC binding capacity and/or by T cell recognition.

MHC binding motif

Peptides associated to a particular MHC molecule (allelic variant) have common structural characteristics, denoted as binding motifs, necessary to form stable complexes with MHC molecules. Peptide ligands eluted from MHC class I molecules are relatively short, ranging from 8-11 amino acids. Moreover, 2 or 3 side chains of the peptide are relevant for binding. The position of the respective amino acid side chains varies with the HLA allele, most often two of these so-called "anchor" residues are located at positions 2 and 9. With respect to a particular anchor position, only 1 or 2 amino acids normally can function as anchor amino acids e.g. leucine or valine V at position 2 in the case of HLA-A2.

In the case of MHC class II molecules, the peptide length varies from 11 to 25 amino acids, as longer peptides can bind since both ends of the peptide binding groove are open. Most HLA class II molecules accommodate up to 4 anchor residues at relative positions P1, P4, P6 and P9 contained in a nonameric core region. This core region, however, can have variable distance from the N-terminus of the peptide. In the majority of cases, 2-4 N-terminal residues precede the core region. Hence, the P1 anchor residues is located at positions 3, 4 or 5 in most HLA class II associated peptides. Peptides eluted from HLA-DR class II molecules share a big hydrophobic P1 anchor, represented by tyrosine, phenylalanine, tryptophane, methionine, leucine, isoleucine or valine.

The position and the exact type of anchor residues constitute the peptide binding motif which is known for most of the frequently occurring HLA class II allelic products. A computer algorithm allowing motif validation in peptide sequences is "Tepitope", available by vaccinome (www.vaccinome.com).

MHC binding capacity

Peptides identified by the method of the invention may be tested for their ability to bind to the appropriate MHC class II molecule by methods known in the art using, for example, isolated MHC class II molecules and synthetic peptides with amino acid sequences identical to those identified by the method of the invention (Kropshofer H et al., J. Exp. Med. 1992; 175, 1799-1803; Vogt AB et al., J. Immunol. 1994; 153, 1665-1673; Sloan VS et al., Nature 1995; 375, 802-806). Alternatively, a cellular binding assay using MHC class II expressing cell lines and biotinylated peptides can be used to verify the identified epitope (Arndt SO et al., EMBO J., 2000; 19, 1241-1251)

In both assays, the relative binding capacity of a peptide is measured by determining the concentration necessary to reduce binding of a labelled reporter peptide by 50%. This value is called IC_{50}. Peptide binding with a reasonable affinity to the relevant HLA class II molecules attain IC_{50} values not exceeding 10-fold the IC_{50} of established reference peptides.

The same binding assays can also be used to test the ability of peptides to bind to alternative class II MHC molecules, i.e., class II MHC molecules other than those from which they were eluted using the method of the invention. The diagnostic methods of the invention using such peptides and therapeutic methods of the invention, using either the peptides or peptides derived from them, can be applied to subjects expressing such alternative class II MHC molecules.

T cell recognition

The epitope verification procedure may involve testing of peptides identified by the method of the invention for their ability to activate CD4+ T cell populations. Peptides with amino acid sequences either identical to those identified in the present invention or corresponding to a core sequence derived from a nested group of peptides identified in the present invention are synthesized. The synthetic peptides are then tested for their ability to activate CD4+ T cells from (a) test subjects expressing the MHC class II molecule of interest and having at least one symptom of the disease; and (b) control subjects expressing the MHC class II molecule of interest and having no symptoms of the disease. Additional control subjects can be those with symptoms of the disease and not expressing the MHC class II molecule of interest.

In some diseases (e.g., those with an autoimmune component) responsiveness in the CD4+ T cells of test subjects but not in CD4+ T cells of the control subjects described in (b) provides confirmatory evidence that the relevant peptide is an epitope that activates CD4+ T cells that can initiate, promote, or exacerbate the relevant disease. In other diseases (e.g., cancer or infectious diseases without an autoimmune component), a similar pattern of responsiveness and non-responsiveness to that described in the previous sentence would indicate that the relevant peptide is an epitope that activates CD4+ T cells that can mediate immunity to the disease or, at least, a decrease in the symptoms of the disease.

CD4+ T cell responses can be measured by a variety of *in vitro* methods known in the art. For example, whole peripheral blood mononuclear cells (PBMC) can be cultured with and without a candidate synthetic peptide and their proliferative responses measured by, e.g., incorporation of [^{3}H]-thymidine into their DNA. That the proliferating T cells are

CD4+ T cells can be tested by either eliminating CD4+ T cells from the PBMC prior to assay or by adding inhibitory antibodies that bind to the CD4+ molecule on the T cells, thereby inhibiting proliferation of the latter. In both cases, the proliferative response will be inhibited only if CD4+ T cells are the proliferating cells. Alternatively, CD4+ T cells can be purified from PBMC and tested for proliferative responses to the peptides in the presence of APC expressing the appropriate MHC class II molecule. Such APC can be B-lymphocytes, monocytes, macrophages, or dendritic cells, or whole PBMC. APC can also be immortalized cell lines derived from B-lymphocytes, monocytes, macrophages, or dendritic cells. The APC can endogenously express the MHC class II molecule of interest or they can express transfected polynucleotides encoding such molecules. In all cases the APC can, prior to the assay, be rendered non-proliferative by treatment with, e.g., ionizing radiation or mitomycin-C.

As an alternative to measuring cell proliferation, cytokine production by the CD4+ T cells can be measured by procedures known to those in art. Cytokines include, without limitation, interleukin-2 (IL-2), interferon-gamma (IFN-gamma), interleukin-4 (IL-4), TNF-alpha, interleukin-6 (IL-6), interleukin-10 (IL-l0), interleukin-12 (IL-12) or TGF-beta. Assays to measure them include, without limitation, ELISA, and bio-assays in which cells responsive to the relevant cytokine are tested for responsiveness (e.g., proliferation) in the presence of a test sample.

Alternatively, cytokine production by CD4+ lymphocytes can be directly visualized by intracellular immunofluorescence staining and flow cytometry.

Moreover, the MHC class II antigenic peptides of the present invention may be used in the diagnosis of RA. Therefore, a further embodiment of the invention is the use of an antigenic peptide according to the present invention as a marker for RA.

Preferably, a MHC class II antigenic peptide comprising (a) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57, or (b) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57 with additional N-and C-terminal flanking sequences of a corresponding sequence selected from the group consisting of SEQ ID NOs. 1 to 39 is used as a marker for RA.

In another embodiment, the antigenic peptides of the invention may be used as response markers to track the efficacy of a therapeutic regime. Essentially, a baseline value for an antigenic peptide can be determined, then a given therapeutic agent is administered,

and the levels of the antigenic peptide are monitored subsequently, whereas a change in the level of the antigenic peptide is indicative of the efficacy of a therapeutic treatment.

Furthermore, the antigenic peptides which are only found in certain stages or phases of a disease, preferably of RA, may be utilized as stage-specific markers. Essentially, the levels of the antigenic peptides which have been linked to a certain disease stage are monitored regularly, thereby providing information about the stage of the disease and its progression.

The invention also includes the use of the polypeptides the RA antigenic peptides are derived from as markers for the diagnosis and monitoring of a disease, preferably of RA, and in particular, of erosive versus non-erosive RA. The rationale for the use of the respective proteins is that DCs reside in most tissues where they capture exogenous antigens via specific receptors and via specialized endocytotic mechanisms (e.g. macropinocytosis) followed by presentation of the processed antigens as peptides on MHC class II molecules. Previous studies have shown that the frequency of a peptide epitope found in the context of MHC class II molecules, e.g. the RA antigenic peptides, in the majority of cases mirrors the abundance of the protein from which this particular peptide was derived from. Therefore, not only the RA antigenc peptides but also the corresponding proteins can serve as markers for RA.

Therefore, in a further embodiment of the present invention, the use of a polypeptide selected from the group consisting of interferon-gamma-inducible lysosomal thiol reductase (SEQ ID NO: 40), apolipoprotein B-100 (SEQ ID NO: 41), inter-alpha-trypsin inhibitor heavy chain H4 (SEQ ID NO: 42), complement C4 (SEQ ID NO: 43), complement C3 (SEQ ID NO: 44), SH3 domain-binding glutamic acid-rich-like protein 3 (SEQ ID NO: 45), interleukin-4-induced protein 1 (SEQ ID NO: 46), hemopexin (SEQ ID NO: 47), Hsc70-interacting protein (SEQ ID NO: 48) as a marker for RA is provided. Preferably, the polypeptide is used as a marker for erosive RA. It is also preferred to use the polypetide as a marker for non-erosive RA. Especially preferred is the use of interleukin-4-induced protein 1 (SEQ ID NO: 46) as a marker for RA. The Fig1 polypeptide has not been known as a marker for RA until now, and is considered as an important candidate marker for RA.

The diagnosis of RA can be made by examining expression and/or composition of a polypeptide or peptide marker for RA, by a variety of methods, including enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. A test sample from an individual is assessed for the presence of an alteration in the expression and/or an alteration in composition of a polypeptide or a

peptide of the present invention. An alteration in expression of a polypeptide or peptide can be, for example, an alteration in the quantitative polypeptide expression (*i.e.*, the amount of polypeptide produced); an alteration in the composition of a polypeptide is an alteration in the qualitative polypeptide expression (*e.g.*, expression of a mutant polypeptide or of a different splicing variant).

Both such alterations (quantitative and qualitative) can also be present. An "alteration" in the polypeptide expression or composition, as used herein, refers to an alteration in expression or composition in a test sample, as compared with the expression or composition of the peptide or polypeptide in a control sample. A control sample is a sample that corresponds to the test sample (e.g., is from the same type of cells), and is from an individual who is not affected by RA. An alteration in the expression or composition of the peptide or polypeptide in the test sample, as compared with the control sample, is indicative of RA or a susceptibility to RA. Various means of examining expression or composition of a peptide or polypeptide of the present invention can be used, including spectroscopy, colorimetry, electrophoresis, isoelectric focusing, and immunoassays (*e.g.*, David *et al.*, U.S. Pat. No. 4,376,110) such as immunoblotting (see also Current Protocols in Molecular Biology, particularly chapter 10). For example, in one embodiment, an antibody capable of binding to the polypeptide (*e.g.*, as described above), preferably an antibody with a detectable label, can be used. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

Western blotting analysis, using an antibody as described above that specifically binds to a peptide or polypeptide of the present invention, may be used to measure the level or amount of a peptide or polypeptide in a test sample and comparing it with the level or amount of the peptide or polypeptide in a control sample. Preferably the peptide or polypeptide in a test sample is measured in a homogenous or a heterogenous immuno assay. A level or amount of the polypeptide in the test sample that is higher or lower than the level or amount of the polypeptide in the control sample, such that the difference is statistically significant, is indicative of an alteration in the expression of the polypeptide, and is diagnostic for a RA or a susceptibility to RA.

Therefore, the present invention also relates to a diagnostic composition comprising an antibody reactive with a MHC class II antigenic peptide of the invention.

In a further embodiment the antigenic peptides of the invention or the proteins they are derived from may be used in the prevention and treatment of a disease, preferably of RA.

One aspect of the invention is a therapeutic purpose, wherein one or more of the identified antigenic peptides are used to vaccinate patients against RA, preferably against erosive and/or non-erosive RA. In the course of the vaccination the antigenic peptide would induce an antigen-specific T cell tolerance in the patient which would ultimately lead to regression of the disease or to an attenuation of disease development.

A promising strategy to induce specific immune tolerance in future clinical trials is the use of DNA tolerizing vaccines. DNA tolerizing vaccines encoding autoantigens alone were shown to reduce T cell proliferative responses (Ruiz, P. et al., J Immunol 162 (1999) 3336-3341), while DNA tolerizing vaccines co-delivering autoantigen plus IL-4 also induced protective T_H2 responses (Garren, H. et al., Immunity 15 (2001) 15-22). Examples of non-polynucleotide-specific tolerizing therapies under development include protein antigens, naturally processed peptides, altered peptide ligands, other biomolecules, such as DNA, or proteins and peptides containing posttranslational modifications, and antigens delivered orally to induce "oral tolerance" (reviewed in: Robinson, W.H. et al., Clin Immunol 103 (2002) 7-12). A potential adverse effect with regard to tolerizing therapies is the development of autoimmunity.

To this end, the relevant RA antigenic peptides may be directly administered to the patient in an amount sufficient for the peptides to bind to the MHC molecules, and provoke peripheral tolerance of T cells.

Alternatively, the antigenic peptides of the invention may be utilized for the generation of vaccines based on DCs. In this case, autologous DCs derived from patients' monocytes may be pulsed with the relevant peptides or recombinant proteins containing the relevant peptide sequences.

Therefore, the present invention provides a pharmaceutical composition comprising a MHC class II antigenic peptide comprising (a) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57, or (b) at least the amino acid sequence of the peptide binding motif selected from the group

consisting of SEQ ID NOs. 49 to 57 with additional N-and C-terminal flanking sequences of a corresponding sequence selected from the group consisting of SEQ ID NOs. 1 to 39, an antibody reactive with said antigenic peptide, or a polypeptide selected from the group consisting of SEQ ID NOs 40 to 48, and optionally a pharmaceutically acceptable excipient, diluent or carrier. The antigenic peptide has to be present in an amount sufficient to tolerize specific lymphocytes. Such an amount will depend on the peptide used, the administration, the severity of the disease to be treated and the general conditions of the patient and will usually range from 1 to 50 mg/ml, for example in case of peptides being loaded on dendritic cells.

An acceptable excipient, diluent or carrier may be phosphate buffered saline for *in vitro* studies and physiological salt solutions for *in vivo* applications.

"Vaccination" herein means both active immunization, i. e. the *in vivo* administration of the peptides to elicit an *in vivo* immune tolerance directly in the patient and passive immunization, i. e. the use of the peptides to tolerize *in vitro* CD4+ T lymphocytes or to stimulate autologous or allogeneic dendritic cells, which are subsequently re-inoculated into the patient.

The present invention also provides the antigenic peptides, antibodies, nucleic acids, host cells, methods, compositions and uses substantially as herein before described especially with reference to the Examples.

Having now generally described this invention, the same will become better understood by reference to the specific examples, which are included herein for purpose of illustration only and are not intended to be limiting unless otherwise specified, in connection with the following figures.

Examples

The examples below are illustrated in connection with the figures described above and based on the methodology summarized in Fig 1, as described in the following. Commercially available reagents referred to in the examples, were used according to manufacturer's instructions unless otherwise indicated.

Methodology of the invention

Dendritic cells and culturing

The study was performed with human dendritic cells which were differentiated from monocytes, as described below. Monocytes were purified from human peripheral blood. The blood was taken from healthy donors with the following haplotypes: (1) *HLA-DRB1*0401, *03011*, (2) *HLA-DRB1*0401, *0304*, (3) *HLA-DRB1*0401, *1301*, (4) *HLA-DRB1*0401, *0701*.

All cells were cultured in RPMI 1640 medium (short: RPMI) supplemented with 1 mM Pyruvate, 2 mM Glutamine and 10% heat-inactivated fetal calf serum (Gibco BRL, Rockville, MD).

Isolation of peripheral blood mononuclear cells (PBMCs)

Peripheral blood was obtained from the blood bank in Mannheim, Germany as standard buffy coat preparations from healthy donors. Heparin (200 I.U./ml blood, Liquemine, Roche) was used to prevent clotting. Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation in LSM® (1.077-1.080 g/ml; ICN, Aurora, OH) at 800g (room temperature) for 30 min. PBMCs were collected from the interphase and washed twice in RPMI containing 20 mM Hepes (500g for 15 min, 300g for 5 min). In order to remove erythrocytes, PBMCs were treated with ALT buffer (140 mM ammonium chloride, 20 mM Tris, pH 7.2) for 3 min at 37°C. PBMCs were washed twice with RPMI containing 20 mM Hepes (200g for 5 min).

Generation of dendritic cells from peripheral blood monocytes.

Monocytes were isolated from PBMCs by positive sorting using anti-CD14 magnetic beads (Miltenyi Biotech, Auburn, CA) according to the manufacturer's protocol. Monocytes were cultured in RPMI supplemented with 1% non-essential amino acids (Gibco, BRL, Rockville, MD), 50 ng/ml recombinant human granulocyte macrophage-colony stimulating factor (GM-CSF; S.A. 1.1×10^7 U/mg) (Leucomax; Novartis, Basel

Switzerland) and 3 ng/ml recombinant human IL-4 (S.A. $2.9x10^4$ U/μg) (R&D Systems, Minneapolis, MN). Monocytes were seeded at 0.3 x 10^6/ml in 6-well plates (Costar) for 5 days to obtain immature dendritic cells.

The quality of monocyte-derived immature dendritic cells was routinely monitored by flow-cytometric analysis and assessed to be appropriate when they displayed the following phenotype: CD1a (high), CD3 (neg.), CD14 (low), CD19 (neg.), CD56 (neg.), CD80 (low), CD83 (neg.), CD86 (low) and HLA-DR (high). In contrast, mature dendritic cells (cf. below) display the following phenotype: CD1a (low), CD80 (high), CD83 (high), CD86 (high) and HLA-DR (high). Monoclonal antibodies against CD1a, CD3, CD14, CD19, CD56, CD80, CD83, CD86 as well as the respective isotype controls were purchased from Pharmingen (San Diego, CA).

Exposure of dendritic cells to serum or synovial fluid

Serum and synovial fluid were irradiated for 30 min with ^{137}Cs (70 TBq). To feed dendritic cells with serum- or synovia-derived antigen, 6 x 10^6 immature dendritic cells were pulsed with either 1 ml serum or 0.6 ml synovial fluid. At the same time maturation of dendritic cells was induced by adding 10 ng/ml recombinant human tumor necrosis factor alpha (TNFα; S.A. $1.1x10^5$ U/μg). As a control, 6 x 10^6 immature dendritic cells were incubated with TNFα alone.

After 24 hrs in culture, mature dendritic cells were harvested by centrifugation at 300g for 10 min. Cells were washed with PBS and transferred to an eppendorf tube. After centrifugation at 400g for 3 min, the supernatant was completely removed and the cells were frozen at -70°C.

Generation of anti-HLA class II beads

The anti-HLA-DR monoclonal antibody (mAb) L243 (ATCC, Manassas, VA) was produced by culturing the respective mouse hybridoma cell line. mAb L243 was purified using ProteinA sepharose (Pharmacia, Uppsala, Sweden) and immobilized to CNBr-activated sepharose beads (Pharmacia) at a final concentration of 2.5 mg/ml, according to the manufacturer's protocol. L243 beads were stored in PBS containing 0.1% Zwittergent 3-12 (Calbiochem, La Jolla, CA).

Nano-scale purification of HLA-DR- peptide complexes

Pellets of frozen dendritic cells were resuspended in 10-fold volume of ice cold lysis buffer (1% Triton-X-100, 20 mM Tris, pH 7.8, 5 mM $MgCl_2$, containing protease inhibitors chymostatin, pepstatin, PMSF and leupeptin (Roche, Mannheim, Germany)) and lysed in a horizontal shaker at 1000 rpm, 4°C for 1 h. The cell lysate was cleared from cell debris and nuclei by centrifugation at 10000g, 4°C for 10 min. The lysate was co-incubated with L243 beads (5-10 µl L243 beads per 100 µl cell lysate) in a horizontal shaker at 1000 rpm, 4°C for 2 hrs. Immunoprecipitated HLA-DR-peptide complexes bound to L243 beads were sedimented by centrifugation at 1000g, 4°C for 1 min and washed four times with 500 µl 0.1% Zwittergent 3-12 (Calbiochem) in PBS.

The efficacy of depletion of HLA-DR-peptide complexes was monitored by analyzing the respective cell lysates before and after immunoprecipitation and aliquots of the beads by western blotting using the anti-HLA-DRα-specific mAb 1B5 (Adams, T.E. et al., Immunology 50 (1983) 613-624).

Elution of HLA-DR-associated peptides

HLA-DR-peptide complexes bound to L243 beads were resuspended in 100 µl H_2O (HPLC-grade; Merck, Darmstadt, Germany), transferred to an ultrafiltration tube, Ultrafree MC, 30 kD cut-off (Millipore, Bedford, MA) and washed 10 times with 100 µl H_2O (HPLC-grade) by centrifugation for 1-2 min at 10000g at RT. For eluting the bound peptides, 60 µl 0.1% trifluoracetic acid (Fluka, Buchs, Switzerland) in H_2O (HPLC-grade) was added and incubation was performed for 30 min at 37°C. Eluted peptides were collected in a new eppendorf tube by centrigugation of the Ultrafree unit at 10000g for 3 min at RT and immediately lyophilized in a Speed-Vac™ vacuum centrifuge.

Fractionation by two-dimensional nanoflow LC

To perform high-throughput sequencing of complex peptide mixtures, the MudPIT (multidimensional protein identification technology) was used (Washburn, M.P. et al., Nat Biotechnol 19 (2001), 242-247) which is based on liquid chromatographic fractionation followed by mass spectrometric sequence determination.

To this end, lyophilized peptides eluted from HLA molecules were resuspended in a buffer containing 5% (v/v) acetonitrile (ACN), 0.5% (v/v) acetic acid, 0.012% (v/v) heptafluoro butyric acid (HFBA) and 1% (v/v) formic acid. The peptide mixture was fractionated on a fused-silica microcapillary column (100 µm i.d. × 375 µm) generated by a Model P-2000 laser puller (Sutter Instrument Co., Novato, CA). The microcolumn was packed with 3 µm / C18 reversed-phase material (C18-ACE 3 µm [ProntoSIL 120-3-C18

ACE-EPS, Leonberg, Germany]) followed by 3 cm of 5 µm cation exchange material (Partisphere SCX; Whatman, Clifton, USA).

A fully automated 8-step gradient separation on a LC Packings UltiMate HPLC (LC Packings, San Francisco, USA) was carried out, using the following buffers: 5% ACN / 0.012% HFBA / 0.5% acetic acid (buffer A), 80% ACN / 0.012% HFBA / 0.5% acetic acid (buffer B), 250 mM ammonium acetate / 5% ACN / 0.012% HFBA / 0.5% acetic acid (buffer C), and 1.5 M ammonium acetate / 5% ACN / 0.012% HFBA / 0.5% acetic acid (buffer D). The first 116 min step consisted of a 75 min gradient from 0 to 40% buffer B followed by a 10 min gradient from 40 to 80% buffer B, a 6 min hold at 80% buffer B and a 10 min equilibration step with 100% buffer A. The next 5 steps (146 min each) were characterized by the following profile: 5 min 100% buffer A, 5 min gradient from 0 to x% buffer C, 5 min 100% buffer A, 30 min gradient from 0 to 10% buffer B, 55 min gradient from 10 to 35% buffer B, 20 min gradient from 35 to 50% buffer B, 10 min gradient from 50 to 80% buffer B; a 6 min hold at 80% buffer B, and a 10 min equilibration step with 100% buffer A. The buffer C percentages (x) in steps 2-6 were as follows: 20, 40, 60, 80, and 90%. The 30 min gradient from 0 to 10% buffer B, which is the first linear elution step from the reversed-phase material, was needed in order to sufficiently separate peptide elution from the elution of a major contaminant (m/z=945) which otherwise would have led to the loss of the more hydrophilic peptide peaks. Step 7 consisted of the following profile: 5 min 100% buffer A, 20 min 100% buffer C, 5 min gradient from 0 to 10% buffer B, 35 min gradient from 10 to 35% buffer B, 50 min gradient from 35 to 50% buffer B, 10 min gradient from 50 to 80% buffer B, a 5 min hold at 80% buffer B and a 10 min equilibration step with 100% buffer A. Step 8 was identical to step 7 with the exception of using buffer D instead of buffer C.

Ion trap MS/MS mass spectrometry

The HPLC column was directly coupled to a Finnigan LCQ Deca XP Plus ion trap mass spectrometer (Thermo Finnigan, San Jose, USA) equipped with a nano-LC electrospray ionization source. Mass spectrometry in the MS/MS mode was performed according to the manufacturer's protocol. Peptides were identified by the SEQUEST algorithm (U.S. patents 6,017,693 and 5,538,897).

MALDI-TOF mass spectrometry

Peptides spotted onto an AnchorChip plate were co-cristallized with matrix (5 mg/ml; α-cyano-4-hydroxy-cinnamic acid (Merck, Darmstadt, Germany), 50% acetonitrile, 0.1% trifluoroacetic acid). For qualitative analysis of the whole peptide repertoire, samples were analyzed on an Ultraflex™ MALDI-TOF mass spectrometer (Bruker, Bremen, Germany), according to the manufacturer's protocol.

Sequence identification by SEQUEST and differential dataset analysis.

MS/MS fragmentation data were analyzed with the software SEQUEST (Thermo Finnigan, San Jose, USA). From an in-house protein database, which was created based on the public databases Swiss-Prot and TrEMBL, SEQUEST extracted for each spectrum all peptide sequences that corresponded to the molecular mass of the parent ion and measured the degree of similarity between the experimental spectrum and the theoretical, *in silico* generated, spectrum. Only the top-scoring candidate sequence was listed.

The peptide sequences derived from the SEQUEST analysis and their accompanying information on mass accuracy, scoring parameters and peptide origin were stored in an appropriately designed relational database and further processed. Certain constraints were enforced in order to guarantee the storage of only significant sequences with satisfying SEQUEST scores. The two most important constraints were: (i) keep only those sequences that have a cross correlation coefficient (CC) higher than a certain value and (ii) from the remaining sequences keep those ones which have a predefined delta cross correlation coefficient (ΔCC). For both criteria the minimum chosen values are based on empirical knowledge of interpreting SEQUEST results.

A dataset was defined as the sum of data from a particular set of spectra. The design of database and software allowed queries on a single dataset as well as comparisons of multiple datasets. Such a database and software design enables comparative sample analysis, which is not provided by SEQUEST. For instance, possible queries on a single dataset could provide information on the score distribution among the stored spectra, on the existence of further sequence length variants or common subsequences, or on the protein origin of peptide sequences. Since the occurrence of truncation variants of the same epitope is a general characteristic of class II MHC-bound peptides, the existence of length variants in a dataset provides additional strong evidence for the presence of an epitope in a set of spectra.

The most important feature in the analysis of multiple datasets is the possibility to extract a common subset of sequences that satisfies a given criterion. Such a criterion could

be based on sequence similarity, e.g., within all sequences of a collection of datasets, those sequences were selected that had at least one subsequence in common with any other sequence. Such comparisons across different datasets constitute the differential approach (RA samples versus control samples) and thereby optimize the search for candidate RA marker peptides.

The pairwise similarity scores between sequences were calculated by a software routine, which is an implementation of a standard string-comparison algorithm. Subsequently, these scores were used to group closely-related sequences (sequences sharing a common subsequence) in well-separated clusters by an additionally developed software routine, which is based on a well-established algorithm (hierarchical clustering, UPGMA).

The generated clusters (e.g. of peptide truncation variants) were then used to identify closely-related sequences across different datasets.

Overall, the data evaluation software provided the ability to perform swiftly and reproducibly the following:

-Select from the sequence output generated by SEQUEST those sequences that satisfy reliable empirical criteria.

- Store the data in a database appropriately designed for the discovery process at hand.

-Extract information about the sequence content of each stored dataset. This information is valuable in assessing the importance of individual sequences within the given dataset and, consequently, across multiple datasets.

-Provide, by virtue of the multiple dataset comparisons, a tool that realizes the differential approach, namely the study of the actual sequence content of one sample versus other(s).

Example 1

In this example, the technique mentioned in Figure 1 was used to identify novel HLA-DR-associated peptide markers derived from serum and synovial fluid of patients with non-erosive RA.

6×10^6 immature dendritic cells were pulsed with either 1 ml serum (3 samples) or 0.6 ml synovial fluid (2 samples) of patients with non-erosive RA and cultured for 24 hrs in the presence of 10 ng/ml TNFα. As a control, 6×10^6 dendritic cells were cultured in the presence of TNFα (10 ng/ml) without adding serum but 1 ml of PBS. In an additional experiment 6×10^6 dendritic cells were pulsed with 1 ml serum from 2 healthy test persons and cultured for 24 hrs in the presence of TNFα (10 ng/ml).

Dendritic cells were lysed in detergent TX-100 and HLA-DR molecules were isolated using mAb L243. HLA-DR-associated peptides were eluted with 0.1% TFA and analyzed by high-throughput 2D-LC-MS/MS technology. Peptide identification was achieved by using the SEQUEST algorithm. The peptide sequences derived from the SEQUEST analysis and accompanying information on mass accuracy, scoring parameters and peptide origin were stored in a database and further processed.

The peptide sequences identified from unpulsed DCs (control 1) and from DCs pulsed with the serum of healthy test persons (control 2) were compared with the peptide sequences identified from DCs pulsed with the serum of non-erosive RA patients. Among the RA-specific sequences, only those peptides were selected for further evaluation that re-occurred in at least three of five non-erosive RA samples.

In each serum sample roughly 600±150 individual peptide sequences (cross correlation coefficient $CC > 3.0$ and $\Delta CC > 0.15$) were identified. In the synovia samples the number of individual peptide sequences was slightly smaller (400±30). Approximately 80-85% of the peptides found in RA samples were also identified in control samples, underlining the high reproducibility of the analysis. In the majority of cases, several length variants of the same epitope could be identified which is a typical characteristic of class II MHC-bound antigens and supports the validity of the results (Jones, E.Y., Curr Opin Immunol 9 (1997) 75-79). Further confidence in the quality of the data relies on the fact that several of the identified peptides or proteins have already been described in the context of MHC class II molecules: epitopes derived from ubiquitous proteins like Hsp70, enolase, annexin II, cathepsin C or collagen II, as well as from MHC molecules (HLA-A, -B, -C, -E, -G, and β_2-microglobulin) and CLIP (Chicz, R.M. et al., J Exp Med 178 (1993) 27-47; Sinigaglia, F. & Hammer, J., Curr Opin Immunol 6 (1994) 52-56; Arnold-Schild, D.

et al., J Immunol 162 (1999) 3757-3760; Vogt, A.B. & Kropshofer, H., Trends Biochem Sci 4 (1999) 150-154) were frequently detected.

RA-specific peptide sequences were further validated with regard to binding to the RA susceptibility allele *DRB1*0401* by using the TEPITOPE software (Hammer, J. et al., Adv Immunol 66 (1997) 67-100). This software provides means for the qualitative and quantitative prediction of T cell epitopes.

The output of the study consists of an epitope that occurred, apart from one exception, only in non-erosive RA samples (Table 1).

Interferon-gamma-inducible lysosomal thiol reductase

A very interesting epitope which was identified in 3 out of 5 non-erosive RA samples from serum and synovia is derived from the interferon-gamma-inducible lysosomal thiol reductase (GILT): the 16-mer GILT (192-207) with the amino acid sequence of SEQ ID NO: 3 (Table 1). Further length variants in three other samples support the relevance of this epitope (Table 1): the 14-mer GILT (192-205; SEQ ID NO: 1) and the 17-mer GILT (192-208; SEQ ID NO: 2).

As judged from the shortest length variant, GILT (192-205), the epitope contains a suitable binding motif, with regard to binding to the RA susceptibility allele *DRB1*0401*: 196M serves as a P1 anchor, 199M as a P4 anchor and 201A as a P6 anchor. According to TEPITOPE scoring, the epitope has a binding score (threshold value) of 1% which is similar to the binding score of an epitope from influenza haemagglutinin (307-319) that was shown to be a strong *DRB1*0401* binder (Table 1) (Rothbard, J.B. et al., Cell 52 (1988) 515-523).

GILT is constitutively expressed in antigen-presenting cells, such as dendritic cells, macrophages and B cells, and facilitates unfolding of endocytosed antigens in MHC class II-containing compartments (MIIC) by enzymatically reducing disulfide bonds (Phan, U.T. et al., J Biol Chem 275 (2000) 25907-25914). Direct binding of GILT to HLA-DR molecules has been reported for B cells (Arunachalam, B. et al., J Immunol 160 (1998) 5797-5806). A rather long second epitope of GILT was found to bind to HLA-DR3 molecules: the 22-mer GILT (38-59) having the amino acid sequence SPLQALDFFGNGPPVNYKTGNL (Chicz, R.M. et al., J Exp Med 178 (1993) 27-47).

In addition to GILT (192-207), another epitope of the same protein was identified in several RA samples, but also in control samples: GILT (210-227) with the amino acid

sequence QPPHEYVPWVTVNGKPLE. This epitope was accompanied by 3 other length variants: the 16-mer GILT (210-225), the 17-mer GILT (210-226) and the 19-mer GILT (210-228).

As indicated by its name, GILT expression can be induced by the pro-inflammatory cytokine interferon gamma (IFN-γ) in various types of cells, including macrophages, endothelial cells and fibroblasts (Luster, A.D. et al., J Biol Chem 263 (1988) 12036-12043). As IFN-γ is known to be present in inflamed joints of RA patients, GILT could become over-expressed in synovia and serum and, hence, could be taken up by DCs as an exogenous antigen. GILT (192-207) may be derived from exogenous GILT. The other GILT epitope, which is also present in the control samples, may be derived from endogenous GILT, expressed by DCs. Alternatively, both GILT (192-207) and GILT (210-227) may be derived from endogenous GILT, in case that GILT processing and GILT-derived epitope presentation by DCs were critically altered upon contact with RA-associated material.

Example 2

In this example, the same technology was used that has been described in detail in example 1. Serum (4 samples) and synovial fluid (2 samples) of patients with diagnosed erosive RA were utilized in this case to identify candidate markers specific for erosive RA.

The peptide sequences found in the erosive RA samples were compared with the sequences identified in unpulsed DCs (control 1) and in DCs pulsed with the serum of healthy test persons (control 2). Among the RA-specific sequences, only those peptides were selected for further evaluation that re-occurred in at least three of six erosive RA samples.

In this study one epitope was discovered which occurred, apart from one exception, only in erosive RA samples.

Apolipoprotein B-100

The epitope which was mainly found in erosive RA sera (4 out of 6 erosive RA samples) is derived from apolipoprotein B-100: the 16-mer ApoB (2877-2892) with the amino acid sequence of SEQ ID NO: 4 (Table 2). In addition a length variant of the same epitope was identified (Table 2): the 17-mer ApoB (2877-2893; SEQ ID NO: 5). The

following *DRB1*0401* binding motif can be predicted: 2881L as a P1 anchor, 2884D as a P4 anchor and 2886N as a P6 anchor (binding score 3%).

In an earlier study on EBV-B cells, the epitope ApoB (2885-2900), which partly overlaps with the epitope described here, has been found in the context of HLA-DR4 (Chicz, R.M. et al., J Exp Med 178 (1993) 27-47).

Apolipoprotein B-100 is a constituent of very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) and functions as a recognition signal for the cellular binding and internalization of LDL particles by the ApoB/E receptor (Yang, C.Y. et al., Nature 323 (1986) 738-742). Interestingly, an increased ratio of LDL cholesterol to HDL cholesterol was observed among newly diagnosed RA patients (Park, Y.B. et al., J Rheumatol 26 (1999) 1701-1704). The adverse lipid profile in active RA could be improved by treating RA patients with DMARDs without the use of lipid-lowering agents (Park, Y.B. et al., Am J Med 113 (2002) 188-193). Since an increased cardiovascular mortality among patients with chronic inflammatory diseases, such as RA, is well documented (Symmons, D.P. et al., J Rheumatol 25 (1998) 1072-1077) it was suggested that local inflammation in RA leads to altered blood lipid levels, thereby increasing the risk of atherosclerosis. The question whether components of the lipoprotein metabolism are causal for pathogenesis or merely affected by ongoing immune reactions during RA development cannot be answered yet. However, the observation of adverse lipid profiles in RA patients supports the validity of the presented ApoB epitope as a serum-derived RA candidate marker.

The length variant ApoB (2877-2892), but not ApoB (2877-2893), has been identified in samples of two healthy controls (Table 2). Since Apolipoprotein B constitutes 1% of all plasma proteins, the presence of ApoB epitopes in healthy control samples is not surprising. The results suggest that only the length variant ApoB (2877-2893; SEQ ID NO: 5) is specific for erosive RA.

Example 3

All peptide sequences identified in examples 1 and 2 from non-erosive and erosive RA samples were used in this example to search for common markers relevant for both RA types. The RA-specific sequences were again compared with peptide sequences of the control samples (unpulsed DCs and DCs pulsed with the serum of two healthy test persons) and only those peptides were selected for further evaluation that re-occurred in at least three of altogether eleven RA samples (erosive and non-erosive RA).

Inter-alpha-trypsin inhibitor

Six out of seven serum samples (erosive & non-erosive RA) but not any of the controls gave rise to an epitope derived from the heavy chain H4 of the inter-alpha-trypsin inhibitor: ITIH4 (271-287) with the amino acid sequence of SEQ ID NO: 8 (Table 3). Apart from this major length variant of the ITIH4 epitope, six length variants of the same ITIH4 epitope could be identified (Table 3): the 19-mer ITIH4 (271-289; SEQ ID NO: 6), the 18-mer ITIH4 (271-288; SEQ ID NO: 7), the 16-mer ITIH4 (274-289; SEQ ID NO: 12), the 15-mer ITIH4 (273-287; SEQ ID NO: 10), the 15-mer (274-288; SEQ ID NO: 11) and the 14-mer ITIH4 (274-287; SEQ ID NO: 9).

As judged from the shortest length variant, ITIH4 (274-287), the epitope contains a very strong binding motif, with regard to binding to the RA susceptibility allele *DRB1*0401*: 277F serves as a P1 anchor, 280D as a P4 anchor and 282S as a P6 anchor (binding score: 1%).

ITIH4 belongs to the Inter-alpha-inhibitor (IαI) family which is a group of serum protease inhibitors that bind to hyaluronic acid (HA) and appear to be involved in acute-phase reactions (Salier, J.P. et al., Biochemical Journal 315 (1996) 1-9).

HA is a polysaccharide found in all tissues of the body, in particular, in loose connective tissue, e.g. joint fluid (Evered, D. & Whelan, J. eds., The Biology of Hyaluronan, John Wiley & Sons (1989)). HA has an important structural function in cartilage and other tissues where it stabilizes the extracellular matrix by forming aggregates with proteoglycans. It has also been assigned important biological functions by regulating cellular activities via binding to cell surface proteins, such as CD44 and ICAM-1 (Knudson, C.B. & Knudson, W., FASEB J 7 (1993) 1233-1241; Hall, C.L. et al., J Cell Biol 126 (1994) 575-588). RA is accompanied by a large increase in total HA in the joint fluid as well as in the serum, suggesting that circulating HA originates from rheumatoid joints (Engström-Laurent, A. et al., Scand J Clin Lab Invest 45 (1985) 497-504).

Complexes of HA and some IαI family members were observed in large amounts in the synovial fluid of RA patients (Jessen, T.E. et al., Biological Chemistry Hoppe-Seyler 375 (1994) 521-526). The role of the IαI-HA complex in inflammatory reactions might be to modify the CD44-HA interaction that mediates leukocyte activation and invasion (Isacke, C.M. & Yarwood, H., Int J Biochem Cell Biol 34 (2002) 718-721). Additionally, synovial fluid of RA patients contains elevated levels of TSG-6, an anti-inflammatory glycoprotein and a member of the hyaladherin family of HA-binding proteins (Wisniewski, H.G. et al., J Immunol 151 (1993) 6593-6601). It has been shown that a complex of TSG-6 with IαI family members inhibits the activity of plasmin, a central molecule in the activation of

inflammation-associated enzymes (Wisniewski, H.G. et al., J Immunol 156 (1996) 1609-1615). A regulation of plasmin activity by several acute-phase plasma proteins, namely TSG-6 and IαI family members, may prove to be important in RA, given the high contents of HA, TSG-6 and IαI family members in synovial fluid of inflamed joints.

This evidence, together with the identification of multiple length variants of the same epitope and a strong HLA-DR4 binding motif, convincingly support the validity of the presented ITIH4 epitope as a serum-derived RA candidate marker.

Complement C4

In all erosive and non-erosive RA sera tested, another dominant epitope was identified which is derived from complement C4: the 15-mer C4 (1697-1711) with the amino acid sequence of SEQ ID NO: 13 (Table 3). Five additional length variants of the same epitope could be found (Table 3): the 12-mer C4 (1697-1708; SEQ ID NO: 18), the 13-mer C4 (1698-1710; SEQ ID NO: 17), the 14-mer C4 (1697-1710; SEQ ID NO: 15), the 16-mer C4 (1697-1712; SEQ ID NO: 14) and the 18-mer C4 (1697-1714; SEQ ID NO: 16). Moreover, the presented epitope displays a very strong *DRB1*0401* binding motif: 1700Y as P1 anchor, 1704D as P2 anchor and 1706N as P6 anchor (binding score: 1%).

C4 which constitutes approximately 0.5% of plasma protein mass plays a critical role in the triggering of the central pathway of the complement system. The protein is synthesized as a single-chain precursor and, prior to secretion, is enzymatically cleaved to form a trimer of non-identical α-, β-, and γ-chains. The identified epitope C4 (1697-1711) is located at the very C-terminus of the C4 γ-chain. The C4 α-chain is further proteolytically degraded by activated C1 to form the C4a anaphylatoxin, which is a mediator of local inflammatory processes (Moon, K.E. et al., J Biol Chem 256 (1981) 8685-8692).

In general, the complement cascade is involved in the induction and progression of inflammatory reactions and is a major defense system against various pathogenic agents, including bacteria, viruses and other antigens (Morgan, B.P., Methods Mol Biol 150 (2000) 1-13). Inappropriate activation, however, can lead to tissue damage and manifestation of disease (Speth, C. et al., Wien Klin Wochenschr 111 (1999) 378-391).

Activation of the complement system has been repeatedly implicated in the pathogenesis of RA, based on studies showing increased levels of complement metabolites, including C4 and C4a, in plasma, synovial fluid and synovial tissue of RA patients

(Neumann, E. et al., Arthritis Rheum 46 (2002) 934-945). In addition collagen-induced arthritis (CIA) in mice is characterized by the presence of complement activation products (Linton, S.M. & Morgan, B.P., Mol Immunol 36 (1999) 905-914). CIA is prevented after treatment with anti-C5 monoclonal antibodies (Wang, Y. et al., PNAS 92 (1995) 8955-8959) or with soluble CR1, an inhibitor of the complement system, delivered by gene therapy (Dreja, H. et al., Arthritis Rheum 43 (2000) 1698-1709). Activation of complement factors in joints is possibly induced by the presence of various immune complexes and it was hypothesized that stimulation of the innate immune system by infectious agents and cytokines may contribute to the initiation of RA (Friese, M.A. et al., Clin Exp Immunol 121 (2000) 406-414).

Two of the six presented C4 epitopes, the 15- and the 18-mer, were also identified in healthy control samples (Table 3) indicating that only some length variants of this C4 epitope are RA-specific, namely the antigenic peptides of SEQ ID NOs: 14, 15, 17, and 18.

Complement C3

Another epitope that was found in erosive and non-erosive RA samples is derived from complement C3: the 14-mer C3 (1431-1444) with the amino acid sequence of SEQ ID NO: 21 (Table 3). Four additional length variants of the same epitope were identified in serum (Table 3): the 13-mer C3 (1431-1443; SEQ ID NO: 23), the 15-mer C3 (1431-1445; SEQ ID NO: 22), the 15-mer C3 (1429-1443; SEQ ID NO: 20) and the 19-mer C3 (1426-1444; SEQ ID NO: 19). As judged from the shortest length variant, C3 (1431-1443), a *DRB1*0401* binding motif can be postulated: 1434Y serves as a P1 anchor, 1437D as a P4 anchor and 1439A as a P6 anchor.

Complement C3 which constitutes about 1-2% of plasma protein mass plays a central role in the activation of the complement system and belongs to the family of the acute-phase proteins. Its processing by C3 convertase to C3a anaphylatoxin and C3b is the central step in both the classical and alternative complement pathways (Barrington, R. et al., Immunol Rev 180 (2001) 5-15). After activation, C3b can bind covalently, via a reactive thiolester, to cell surface carbohydrates or immune aggregates (Isaac, L. & Isenman, D.E., J Biol Chem 267 (1992) 10062-10069). The identified epitope C3 (1431-1444) is located at the C-terminus of C3b.

As already discussed in the context of complement epitope C4 (1697-1711), there is increasing evidence for an important role of components of the complement cascade in the pathophysiology of RA. The result of this study, in which two major epitopes derived from

complement C3 and C4 were identified in serum of RA patients, underlines the close link between the activated complement system and pathogenesis of RA. This coincidence makes a strong argument for the validity of the presented C3 / C4 epitopes as serum-derived candidate RA markers.

SH3 domain-binding glutamic acid-rich-like protein 3

Another epitope which was elucidated quite frequently in serum of RA patients (5 out of 7 erosive and non-erosive RA samples), is derived from the SH3 domain-binding glutamic acid-rich-like protein 3 (SH3BGRL3): SH3BGRL3 (15-26) with the amino acid sequence of SEQ ID NO: 25 (Table 3). Three length variants of the same epitope were identified (Table 3): the 14-mer SH3BGRL3 (13-26; SEQ ID NO: 26), the 14-mer SH3BGRL3 (15-28; SEQ ID NO: 27) and the 16-mer SH3BGRL3 (13-28; SEQ ID NO: 24). The *DRB1*0401* binding motif is: 17I as P1 anchor, 20Q as P4 anchor and 22S as P6 anchor (binding score 4%).

SH3BGRL3 is a small 10 kD protein that belongs to the SH3BGR family. The precise function of the protein is unknown but a role as a modulator of glutaredoxin biological activity is postulated (Mazzocco, M. et al., Biochem Biophys Res Commun 285 (2001) 540-545). So far, SH3BGRL3 has not been described in the context of RA.

Interestingly, the analysis elucidated a second epitope of the same protein, which was highly abundant in all RA and control samples: the 16-mer SH3BGRL3 (29-44) with the amino acid sequence DGKRIQYQLVDISQDN. In addition multiple length variants of the same epitope were found in most samples as well. As judged from the shortest length variant, SH3BGRL3 (31-42), the epitope contains almost similar *DRB1*0401* anchor residues compared with SH3BGRL3 (15-26): 33I serves as a P1 anchor, 36Q as a P4 anchor and 38V as a P6 anchor (binding score -2). This similarity is reflected by comparable binding scores.

The presence of this second SH3BGRL3 epitope supports the validity of the SH3BGRL3 (15-26) epitope because both peptides are derived from the same protein, however, only one of them, epitope SH3BGRL3 (15-26), appears to be generated in a RA-specific manner. A similar observation has been described already for GILT in example 1.

Among the four SH3BGRL3 length variants the longest variant, SH3BGRL3 (13-28), was also identified in a healthy control sample (Table 3). However, this particular length variant was found only one time, which indicates a significant enrichment of the SH3BGRL3 epitope in the context of RA.

Interleukin-4 (IL-4) induced protein 1

In all the investigated sera and synovial fluids (erosive & non-erosive RA), one highly dominant epitope was identified which is derived from the human homolog of the IL-4 induced protein 1 (Fig1): Fig1 (293-309) with the amino acid sequence of SEQ ID NO: 28 (Table 3). The validity of the epitope was further supported by the presence of additional length variants in several samples (Table 3): the 16-mer Fig1 (293-308; SEQ ID NO: 30) and the 19-mer Fig1 (293-311; SEQ ID NO: 29). Moreover, the amino acid sequence displays a typical *DRB1*0401* binding motif: 299V serves as P1 anchor, 302E as P4 anchor and 304S as P6 anchor (binding score 1%).

Two length variants of the same epitope, Fig1 (293-308) and Fig1 (293-309), were identified in one unpulsed sample and in one healthy control sample as well (Table 3). However, the presence of the Fig1 epitope in all RA samples but not in all of the control samples tested strongly indicates an enrichment in the context of RA.

The human *fig1* gene was first identified in IL-4-stimulated B cell cultures (Chu, C.C. & Paul, W.E., PNAS 94 (1997) 2507-2512). The human *fig1* resides on chromosome 19q13.3-19q13.4, a region previously identified to be involved in susceptibility to autoimmune diseases, including SLE, arthritis, multiple sclerosis, and insulin-dependent diabetes mellitus (Becker K.G. et al., PNAS 95 (1998) 9979-9984). Since its expression is largely limited to immune tissues and its regulation is dependent on IL-4, a key modulator of the immune response, *fig1* is thus an attractive candidate gene for autoimmune disease susceptibility (Chavan, S.S. et al., Biochim Biophys Acta 1576 (2002) 70-80). The HLA-DR4-restricted presentation of a Fig1 epitope provides the first indication that Fig1 protein is produced and possibly involved in the disease development of RA. The Fig1 polypeptide has not been known as a marker for RA until now, and is considered as an important candidate marker for RA.

Hemopexin

Another RA candidate marker which was frequently identified in serum samples (6 out of 7 samples) and in synovia samples (2 out of 4 samples) (erosive & non-erosive RA) is derived from hemopexin (HPX): HPX (351-367) with the amino acid sequence of SEQ ID NO: 32 (Table 3). Several length variants were found which support the validity of this epitope (Table 3): the 13-mer HPX (351-363; SEQ ID NO: 33), the 14-mer HPX (350-363; SEQ ID NO: 34), the 15-mer HPX (351-365; SEQ ID NO: 35) and the 18-mer HPX (351-368; SEQ ID NO: 31). Furthermore, the epitope contains a very strong *DRB1*0401* binding

motif: 355I serves as a P1 anchor, 358D as a P4 anchor and 360V as a P6 anchor (binding score: 1%).

Two length variants of the same epitope, HPX (351-367; SEQ ID NO: 32) and HPX (351-365; SEQ ID NO: 35), could also be identified in healthy control samples (Table 3) indicating that only some length variants are specific for RA, namely the antigenic peptides of SEQ ID NOs. 31, 33 and 34.

HPX is a 60 kD plasma glycoprotein with a high binding affinity to heme (Müller-Eberhard, U., Methods Enzymol 163 (1988) 536-565). It is mainly expressed in the liver, and belongs to the acute-phase proteins the synthesis of which is induced in an inflammatory situation. RA is a chronic inflammatory autoimmune disease and elevated levels of several acute-phase proteins, including C-reactive protein and serum amyloid A, have been reported (Nakamura, R., J Clin Lab Anal 14 (2000) 305-313). HPX is responsive to the cytokines IL-1 and IL-6, which are upregulated in patients suffering from RA (Feldmann, M. & Maini, R.N., Rheumatology 38, Suppl 2 (1999) 3-7).

HPX is the major vehicle for the transportation of heme in the plasma and its principal role is to prevent heme-mediated oxidative stress and loss of heme-bound iron (Tolosano, E. & Altruda, F., DNA Cell Biol 21 (2002) 297-306). It can protect cells against oxidative stress by inducing the expression of intracellular antioxidants such as heme oxygenase, metallothioneins and ferritin. Metallothioneins are cytosolic proteins that are expressed particularly in synovial fibroblasts (Backman, J.T. et al., Virchows Arch 433 (1998) 153-160). There is significant experimental evidence for the presence of oxidative stress in the synovial tissue of RA patients (reviewed in: Schett, G. et al., Arthritis Res 3 (2000) 80-86). Furthermore HPX was reported to promote proliferation of human T lymphocytes (Smith, A. et al., Exp Cell Res 232 (1997) 246-254). These studies render it likely that HPX belongs to the up-regulated proteins in serum and synovia of RA patients, thereby providing a rationale for the relevance of HPX (351-367) as a RA-specific candidate marker.

Hsc70-interacting protein

An epitope which was mostly identified in serum samples (4 out of 7 erosive and non-erosive RA samples) and which is also related to stress responses is derived from the Hsc70-interacting protein Hip: Hip (83-98) with the amino acid sequence of SEQ ID NO: 38 (Table 3). Two length variants of this epitope were identified (Table 3): the 18-mer Hip (83-100; SEQ ID NO: 36) and the 15-mer Hip (84-98; SEQ ID NO: 39). An additional

length variant was discovered in one erosive synovia sample (Table 3): the 15-mer Hip (85-99; SEQ ID NO: 37). As judged from the shortest length variant Hip (84-98) a *DRB1*0401* binding motif attaining a moderate score of 8%, can be postulated: 89I as P1 anchor, 92D as P4 anchor, 94D as P6 anchor.

In the cytosol of eukaryotic cells, Hip and Hop proteins associate with Hsc70 in order to participate in the regulation of Hsc70 chaperone activity (Frydman, J. & Höhfeld, J., Trends Biochem Sci 22 (1997) 87-92). The 42 kD Hip protein binds to the ATPase domain of Hsc70. It was postulated that Hip might increase the half-life of the chaperone-substrate complex providing the molecular basis for an efficient cooperation of Hsc70 with downstream chaperone systems. Hsc70 and Hsp90 have been shown to cooperate during protein folding *in vitro* (Jakob, U. & Buchner, J., Trends Biochem Sci 19 (1994) 205-211; Freeman, B.C. & Morimoto, R.I., EMBO J 15 (1996) 2969-2979) and to play a role in thermal denaturation (Schneider, C. et al., PNAS 93 (1996) 14536-14541). The Hsc70 and Hsp90 association with stress-adaptation ultimately links Hip to stress responses, including the induction of heat shock proteins, in the synovial tissue of RA patients (reviewed in: Schett, G. et al., Arthritis Res 3 (2001) 80-86).

Table 1: HLA-DR associated peptide antigens from serum and synovial fluid of patients with mostly non-erosive RA.

SEQ. ID. NO.	RA-type[a]	RF[b] (IU/ml)	Sample[c]	Haplo-type[d]	Length	Sequence[e]	*DRB1*0401*-binding score[f]	Protein source[g]
1	N	-	S	1	14	GDRGMQLMHANAQR	1%	Interferon-gamma-inducible lysosomal thiol reductase (192-205)
2	N	6.8	S	3	17	GDRGMQLMHANAQRTDA		
3	N	6.8	S	3	16	GDRGMQLMHANAQRTD		
2	N	9.1	Syn	4	17	GDRGMQLMHANAQRTDA		
3	N	9.1	Syn	4	16	GDRGMQLMHANAQRTD		
3	E	20.7	S	3	16	GDRGMQLMHANAQRTD		
	strong HLA-DRB1*0401 binder					PKYVKQNTLKLAT[h (i)]	1%	Influenza Haemagglutinin (307-319)
	moderate HLA-DRB1*0401 binder					KHKVYACEVTHQGLS[h (ii)]	2%	Immunglobuline kappa (188-202)
	weak HLA-DRB1*0401 binder					KTAAYDEEARR[h (iii)]	> 10%	*M. tuberculosis* Hsp65 (3-13)

[a]RA-type of the patient based on clinical diagnosis: persistant erosive (E) or persistent non-erosive (N) RA

[b]Rheumatoid factor

[c]Sample description: dendritic cells pulsed with serum (S) or synovial fluid (Syn)

[d]Haplotype of the buffy coat: (1) *HLA-DRB1*0401, *03011*; (2) *HLA-DRB1*0401, *0304*; (3) *HLA-DRB1*0401, *1301*; (4) *HLA-DRB1*0401, *0701*

[e]Sequences of the RA-derived peptides in one-letter-code. The *HLA-DRB1*0401* binding motif is boxed in grey.

[f]Score of the epitope in context of the *HLA-DRB1*0401* allele based on the TEPITOPE program (Hammer, J. et al., Adv Immunol 66 (1997) 67-100).

[g]Protein name according to the Swiss-Prot / TrEMBL database. The numbers in brackets represent the shortest length variant of the respective epitope.

[h (i)] Rothbard, J.B. et al., Cell 52 (1988) 515-523. [h (ii)] Chicz, R.M. et al., J Exp Med 178 (1993) 27-47. [h (iii)] van Schooten, W.C. et al., Eur J Immunol 19 (1989) 2075-2079.

Table 2: HLA-DR associated peptide antigens from serum and synovial fluid of patients with mostly erosive RA.

SEQ. ID. NO.	RA-type[a]	RF[b] (IU/ml)	Sample[c]	Haplo-type[d]	Length	Sequence[e]	*DRB1*0401*-binding score[f]	Protein source[g]
4	E	-	S	1	16	INNQLTLDSNTKYFHK**	3%	Apolipoprotein B-100 (2877-2892)
4	E	+	S	2	16	INNQLTLDSNTKYFHK**		
4	E	134	S	3	16	INNQLTLDSNTKYFHK**		
4	E	20.7	S	3	16	INNQLTLDSNTKYFHK**		
5	E	20.7	S	3	17	INNQLTLDSNTKYFHKL		
4	N	-	S	1	16	INNQLTLDSNTKYFHK**		

[a]RA-type of the patient based on clinical diagnosis: persistant erosive (E) or persistent non-erosive (N) RA

[b]Rheumatoid factor

[c]Sample description: dendritic cells pulsed with serum (S) or synovial fluid (Syn)

[d]Haplotype of the buffy coat: (1) *HLA-DRB1*0401, *03011*; (2) *HLA-DRB1*0401, *0304*; (3) *HLA-DRB1*0401, *1301*; (4) *HLA-DRB1*0401, *0701*

[e]Sequences of the RA-derived peptides in one-letter-code. The *HLA-DRB1*0401* binding motif is boxed in grey.

[f]Score of the epitope in context of the *HLA-DRB1*0401* allele based on the TEPITOPE program (Hammer, J. et al., Adv Immunol 66 (1997) 67-100).

[g]Protein name according to the Swiss-Prot / TrEMBL database. The numbers in brackets represent the shortest length variant of the respective epitope.

** Length variant of the respective epitope, which was identified in 2 healthy control samples as well.

Table 3: HLA-DR associated peptide antigens from serum or synvial fluid of patients with erosive and non-erosive RA.

SEQ. ID No.	RA-type[a]	RF[b] (IU/ml)	Sample[c]	Haplo-type[d]	Length	Sequence[e]	*DRB1*0401*-binding score[f]	Protein source[g]
6	E	-	S	3	19	MPKNVVFVIDKSGSMSGRK	1%	Inter-alpha-trypsin inhibitor heavy chain H4 (274-287)
7	E	+	S	3	18	MPKNVVFVIDKSGSMSGR		
8	E	+	S	3	17	MPKNVVFVIDKSGSMSG		
8	E	134	S	3	17	MPKNVVFVIDKSGSMSG		
9	E	134	S	3	14	NVVFVIDKSGSMSG		
8	E	134	S	2	17	MPKNVVFVIDKSGSMSG		
10	E	134	S	2	15	KNVVFVIDKSGSMSG		
8	E	20.7	S	1	17	MPKNVVFVIDKSGSMSG		
11	E	20.7	S	1	15	NVVFVIDKSGSMSGR		
6	N	-	S	3	19	MPKNVVFVIDKSGSMSGRK		
7	N	6.8	S	3	18	MPKNVVFVIDKSGSMSGR		
8	N	6.8	S	3	17	MPKNVVFVIDKSGSMSG		
8	N	6.8	S	3	17	MPKNVVFVIDKSGSMSG		
12	N	6.8	S	3	16	NVVFVIDKSGSMSGRK		
8	N	6.8	S	1	17	MPKNVVFVIDKSGSMSG		
13	N	-	S	1	15	GHPQYLLDSNSWIEE**	1%	Complement C4 (1697-1708)
14	N	-	S	1	16	GHPQYLLDSNSWIEEM		
15	N	-	S	1	14	GHPQYLLDSNSWIE		
13	N	6.8	S	3	15	GHPQYLLDSNSWIEE**		

16	N	9.1	S	4	18	GHPQYLLDSNSWIEEMPS*		
17	N	9.1	S	4	13	HPQYLLDSNSWIE		
13	N	9.1	S	4	15	GHPQYLLDSNSWIEE**		
18	N	9.1	S	4	12	GHPQYLLDSNSW		
14	E	-	S	1	16	GHPQYLLDSNSWIEEM		
13	E	-	S	1	15	GHPQYLLDSNSWIEE**		
18	E	-	S	1	12	GHPQYLLDSNSW		
13	E	+	S	2	15	GHPQYLLDSNSWIEE**		
13	E	134	S	3	15	GHPQYLLDSNSWIEE**		
15	E	134	S	3	14	GHPQYLLDSNSWIE		
16	E	134	S	3	18	GHPQYLLDSNSWIEEMPS*		
13	E	20.7	S	3	15	GHPQYLLDSNSWIEE		
16	E	20.7	S	3	18	GHPQYLLDSNSWIEEMPS*		
19	N	9.1	S	4	19	GVDRYISKYELDKAFSDRN	9%	Complement C3 (1431-1443)
20	N	-	S	1	15	RYISKYELDKAFSDR		
21	N	-	S	1	14	ISKYELDKAFSDRN		
22	N	-	S	1	15	ISKYELDKAFSDRNT		
23	N	-	S	1	13	ISKYELDKAFSDR		
21	E	+	S	2	14	ISKYELDKAFSDRN		
24	N	6.8	S	3	16	GSREIKSQQSEVTRIL*	4%	SH3 domain-binding glutamic acid-rich-like protein 3 (15-26)
25	N	-	S	1	12	REIKSQQSEVTR		
26	N	-	S	1	14	GSREIKSQQSEVTR		
24	N	-	S	1	16	GSREIKSQQSEVTRIL*		

27	N	-	S	1	14	REIKSQQSEVTRIL		
26	E	-	S	1	14	GSREIKSQQSEVTR		
25	E	-	S	1	12	REIKSQQSEVTR		
25	E	134	S	3	12	REIKSQQSEVTR		
25	E	20.7	S	3	12	REIKSQQSEVTR		
24	E	20.7	S	3	16	GSREIKSQQSEVTRIL*		
28	E	20.7	Syn	3	17	GPHDVHVQIETSPPARN	1%	Interleukin-4-induced protein 1 (293-308)
29	E	20.7	Syn	3	19	GPHDVHVQIETSPPARNLK		
29	E	134	Syn	3	19	GPHDVHVQIETSPPARNLK		
28	E	-	S	1	17	GPHDVHVQIETSPPARN		
28	E	+	S	2	17	GPHDVHVQIETSPPARN		
28	E	134	S	3	17	GPHDVHVQIETSPPARN		
30	E	134	S	3	16	GPHDVHVQIETSPPAR*		
30	E	20.7	S	3	16	GPHDVHVQIETSPPAR*		
28	N	6.8	S	3	17	GPHDVHVQIETSPPARN		
28	N	9.1	S	4	17	GPHDVHVQIETSPPARN		
30	N	9.1	S	4	16	GPHDVHVQIETSPPAR*		
28	N	-	S	1	17	GPHDVHVQIETSPPARN		
28	N	9.1	Syn	4	17	GPHDVHVQIETSPPARN		
28	N	6.8	Syn	3	17	GPHDVHVQIETSPPARN		
31	N	-	S	1	18	TPHGIIDSVDAAFICPG	1%	Hemopexin (351-363)
32	N	9.1	S	4	17	TPHGIIDSVDAAFICP**		
33	N	9.1	S	4	13	TPHGIIDSVDAA		

32	N	6.8	Syn	3	17	TPHGIIEDSVDAAFICP**		
32	N	9.1	Syn	4	17	TPHGIIEDSVDAAFICP**		
33	N	9.1	Syn	4	13	TPHGIIEDSVDAA		
34	N	9.1	Syn	4	14	GTPHGIIEDSVDAA		
35	N	6.8	S	3	15	TPHGIIEDSVDAAFI*		
32	N	6.8	S	3	17	TPHGIIEDSVDAAFICP**		
31	E	+	S	2	18	TPHGIIEDSVDAAFICPG		
35	E	134	S	3	15	TPHGIIEDSVDAAFI*		
32	E	134	S	3	17	TPHGIIEDSVDAAFICP**		
35	E	20.7	S	3	15	TPHGIIEDSVDAAFI*		
32	E	20.7	S	3	17	TPHGIIEDSVDAAFICP**		
36	E	134	Syn	3	18	IDKEGVIEPDTDAPQEMG	8%	Hsc70-interacting protein (84-98)
37	E	134	Syn	3	15	KEGVIEPDTDAPQEM		
38	E	-	S	1	16	IDKEGVIEPDTDAPQE		
39	E	-	S	1	15	DKEGVIEPDTDAPQE		
36	N	9.1	S	4	18	IDKEGVIEPDTDAPQEMG		
38	N	-	S	1	16	IDKEGVIEPDTDAPQE		
38	N	6.8	S	3	16	IDKEGVIEPDTDAPQE		

[a]RA-type of the patient based on clinical diagnosis: persistant erosive (E) or persistent non-erosive (N) RA
[b]Rheumatoid factor
[c]Sample description: dendritic cells pulsed with serum (S) or synovial fluid (Syn)
[d]Haplotype of the buffy coat: (1) *HLA-DRB1*0401, *03011*; (2) *HLA-DRB1*0401, *0304*; (3) *HLA-DRB1*0401, *1301*; (4) *HLA-DRB1*0401, *0701*
[e]Sequences of the RA-derived peptides in one-letter-code. The *HLA-DRB1*0401* binding motif is boxed in grey.
[f]Score of the epitope in context of the *HLA-DRB1*0401* allele based on the TEPITOPE program (Hammer, J. et al., Adv Immunol 66 (1997) 67-100).
[g]Protein name according to the Swiss-Prot / TrEMBL database. The numbers in brackets represent the shortest length variant of the respective epitope.
* (**) Length variant of the respective epitope which was identified in 1 (2) healthy control sample(s) as well.

Table 4: Summary of the candidate RA markers.

RA-type	Protein source[a]	Frequency in the RA samples[b]	Accession number[c]
mostly non-erosive	Interferon-gamma-inducible lysosomal thiol reductase	3 of 5 N	P13284
mostly erosive	Apolipoprotein B-100	4 of 6 E	P04114
erosive and non-erosive	Inter-alpha-trypsin inhibitor heavy chain H4	4 of 6 E / 2 of 5 N	Q14624
	Complement C4	4 of 6 E / 3 of 5 N	P01028
	Complement C3	1 of 6 E / 2 of 5 N	P01024
	SH3 domain-binding glutamic acid-rich-like protein 3	3 of 6 E / 2 of 5 N	Q9H299
	Interleukin-4-induced protein 1	6 of 6 E / 5 of 5 N	Q96RQ9
	Hemopexin	3 of 6 E / 5 of 5 N	P02790
	Hsc70-interacting protein	2 of 6 E / 3 of 5 N	P50502

[a]Protein name according to the Swiss-Prot / TrEMBL database.

[b]Frequency of the identified epitope in the RA samples. The RA-type of the patient was based on clinical diagnosis: persistant erosive (E) or persistent non-erosive (N) RA.

[c]relates to the Swiss-Prot database.

Claims

1. A MHC class II antigenic peptide comprising
(a) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57, or
(b) at least the amino acid sequence of the peptide binding motif selected from the group consisting of SEQ ID NOs. 49 to 57 with additional N-and C-terminal flanking sequences of a corresponding sequence selected from the group consisting of SEQ ID NOs. 1 to 39.

2. A MHC class II antigenic peptide comprising
(a) at least the amino acid sequence of the peptide binding motif of SEQ ID NO. 49, or
(b) at least the amino acid sequence of the peptide binding motif of SEQ ID NO. 49 with additional N-and C-terminal flanking sequences of a corresponding sequence selected from the group consisting of SEQ ID NOs. 1 to 3.

3. A MHC class II antigenic peptide comprising
(a) at least the amino acid sequence of the peptide binding motif of SEQ ID NO. 50, or
(b) at least the amino acid sequence of the peptide binding motif of SEQ ID NO. 50 with additional N-and C-terminal flanking sequences of the corresponding sequence of SEQ ID NO. 5.

4. The MHC class II antigenic peptide according to any one of claims 1 to 3 linked to a MHC class II molecule.

5. An antibody reactive with a MHC class II antigenic peptide according to any one of claims 1 to 3.

6. A nucleic acid molecule encoding a peptide or polypeptide according to any one of claims 1 to 4.

7. A recombinant nucleic acid construct comprising the nucleic acid molecule according to claim 6 operably linked to an expression vector.

8. A host cell containing the nucleic acid construct according to claim 7.

9. A method for producing a MHC class II antigenic peptide according to any one of claims 1 to 3 comprising the steps of culturing the host cell of claim 8 under

conditions allowing expression of peptide from the cells or the culture said peptide and recovering the medium.

10. A method for isolating and identifying MHC class II associated RA antigenic peptides in femtomolar amounts, which method comprises
(a) providing immature dendritic cells in a number comprising 0.1 to 5 µg MHC class II molecules;
(b) contacting the cells of (a) with serum or synovial fluid and inducing maturation of dendritic cells by adding TNFalpha;
(c) isolating class II MHC molecule-antigenic peptide complexes from the cells with methods comprising solubilization of the cells and sequestration of the complexes of MHC class II molecules with antigenic peptides by immunoprecipitation or immunoaffinity chromatography;
(d) washing the sequestered complexes of MHC class II molecules with antigenic peptides with water in an ultrafiltration tube;
(e) eluting the associated antigenic peptides from the MHC class II molecules at 37°C with diluted trifluoro acetic acid, and
(f) separating, detecting and identifying the isolated peptides by liquid chromatography and mass spectrometry.

11. The method according to claim 10, wherein in step (f) of the method the liquid chromatography comprises a first linear elution step from the reversed-phase material with a volume sufficient to elute contaminants prior to the peptide elution step.

12. The method according to any one of claims 10 and 11, further comprising
(g) analyzing the identified peptides by methods comprising a database and a software developed to perform comparative data analysis across multiple datasets.

13. A pharmaceutical composition comprising a MHC class II antigenic peptide according to any one of claims 1 to 3, an antibody according to claim 5, or a polypeptide selected from the group consisting of SEQ ID NOs 40 to 48, and optionally a pharmaceutically acceptable carrier.

14. A diagnostic composition comprising the antibody according to claim 5.

15. The use of the MHC class II antigenic peptide according to claim 1, wherein the antigenic peptide is a marker for erosive and/or non-erosive RA.

16. The use of the MHC class II antigenic peptide according to claim 2, wherein the antigenic peptide is a marker for non-erosive RA

17. The use of the MHC class II antigenic peptide according claim 3, wherein the antigenic peptide is a marker for erosive RA.

18. The use of a polypeptide selected from the group consisting of SEQ ID NOs 40 to 48 as a marker for RA, preferably for erosive and/or non-erosive RA.

19. The antigenic peptides, antibodies, nucleic acids, host cells, methods, compositions and uses substantially as herein before described especially with reference to the foregoing Examples.

***

Abstract

The present invention provides novel naturally-processed MHC class II antigenic peptides which originate from interferon-γ-inducible lysosomal thiol reductase, apolipoprotein B-100, inter-α-trypsin inhibitor heavy chain H4, complement C4, complement C3, SH3 domain-binding glutamic acid-rich-like protein 3, interleukin-4-induced protein 1, hemopexin, and Hsc70-interacting protein. Also provided are these antigenic peptides and the proteins they are derived from as markers for erosive and/or non-erosive RA. Moreover, these antigenic peptides linked to MHC class II molecules, antibodies reactive with said antigenic peptides, nucleic acids encoding said antigenic peptides, and nucleic acid constructs, host cells and methods for expressing said antigenic peptides are provided. The antigenic peptides of the invention can be used as markers in diagnosis of RA and in therapy as anti-RA vaccines.

Fig. 1

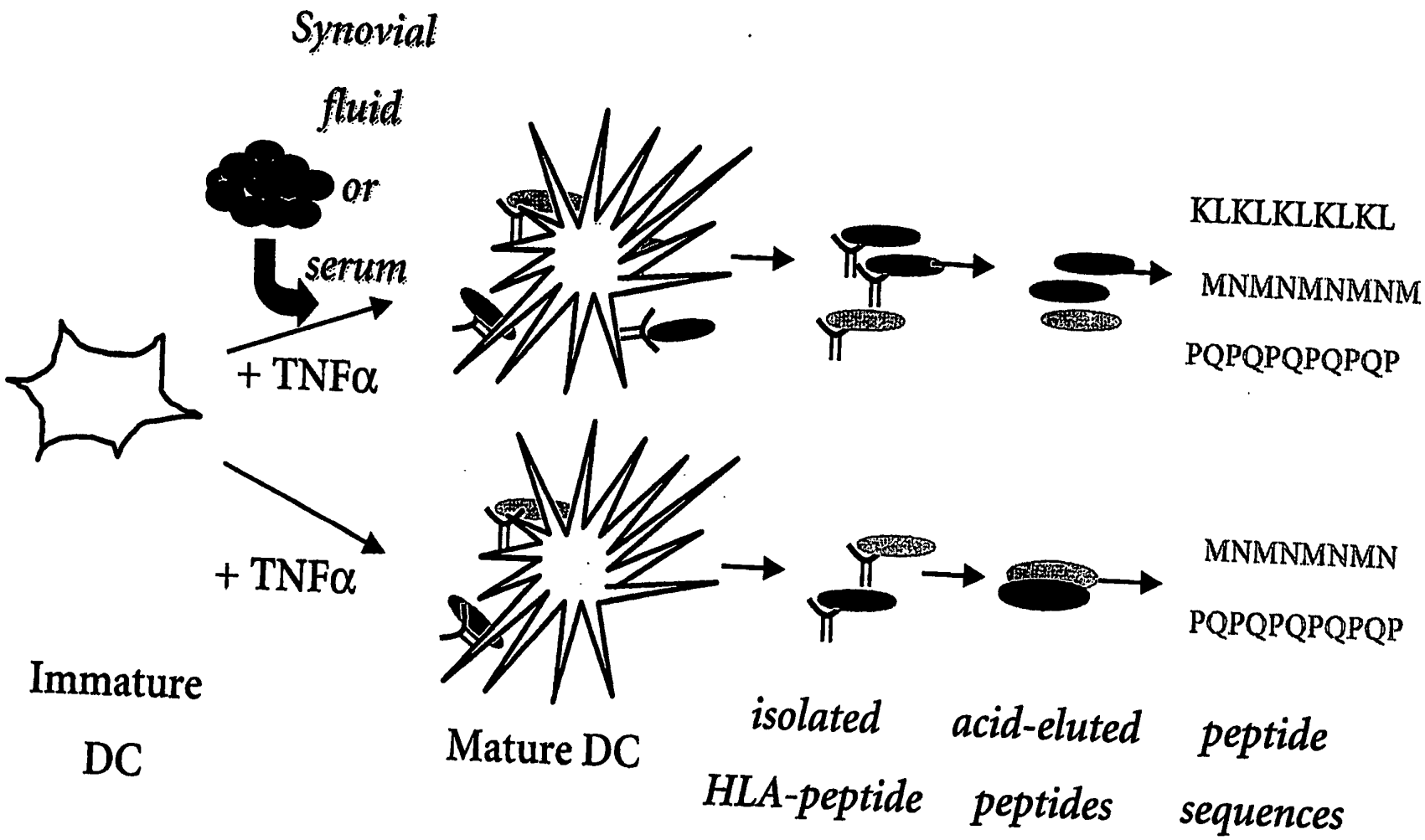

Fig. 2A

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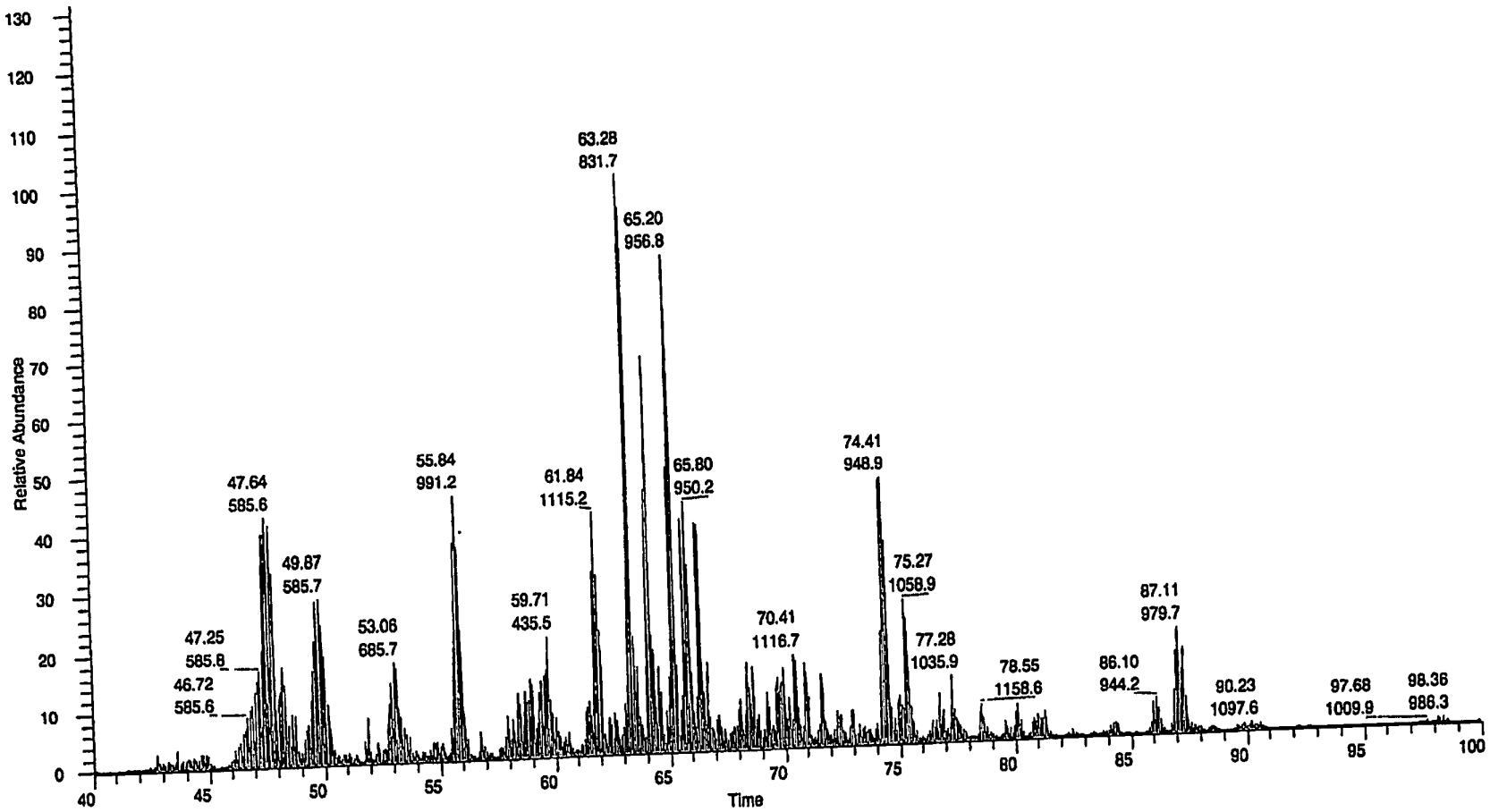
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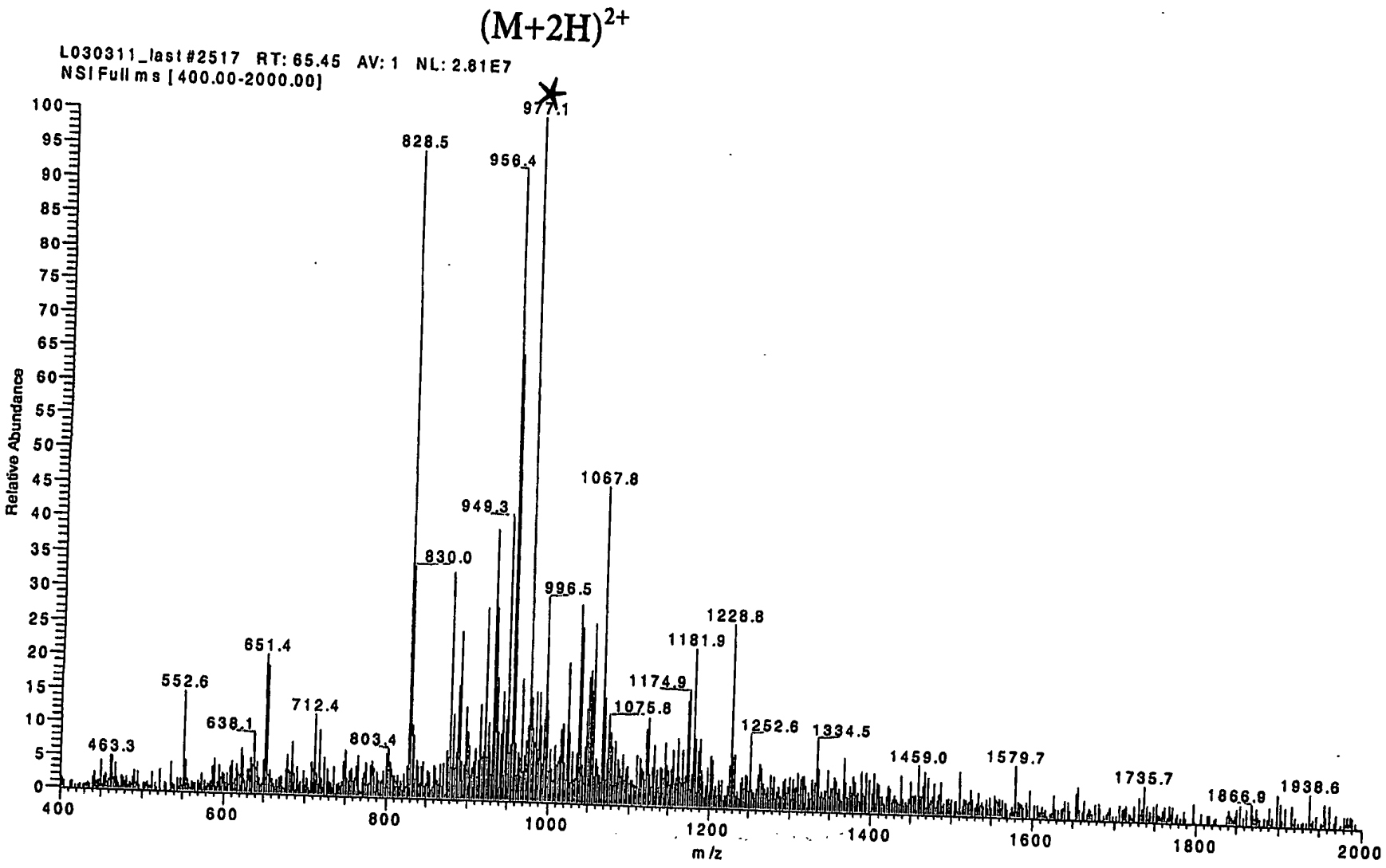
Fig. 2B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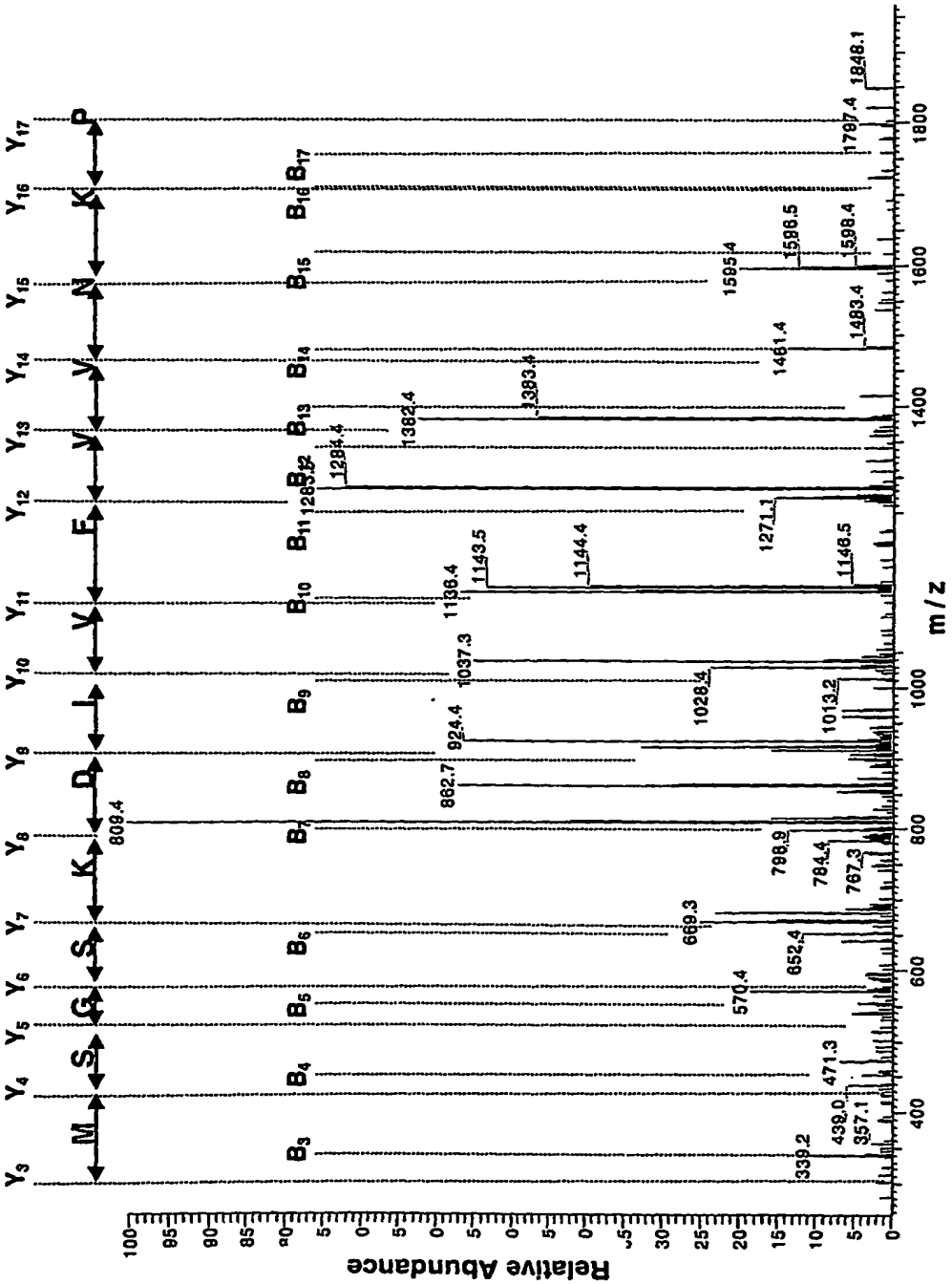
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Fig. 2C

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Asn Arg Gln Ser Met Thr Leu Ser Ser Glu Val Gln Ile Pro Asp
1055 1060 1065

Phe Asp Val Asp Leu Gly Thr Ile Leu Arg Val Asn Asp Glu Ser
1070 1075 1080

Thr Glu Gly Lys Thr Ser Tyr Arg Leu Thr Leu Asp Ile Gln Asn
1085 1090 1095

Lys Lys Ile Thr Glu Val Ala Leu Met Gly His Leu Ser Cys Asp
1100 1105 1110

Thr Lys Glu Glu Arg Lys Ile Lys Gly Val Ile Ser Ile Pro Arg
1115 1120 1125

Leu Gln Ala Glu Ala Arg Ser Glu Ile Leu Ala His Trp Ser Pro
1130 1135 1140

Ala Lys Leu Leu Leu Gln Met Asp Ser Ser Ala Thr Ala Tyr Gly
1145 1150 1155

Ser Thr Val Ser Lys Arg Val Ala Trp His Tyr Asp Glu Glu Lys
1160 1165 1170

Ile Glu Phe Glu Trp Asn Thr Gly Thr Asn Val Asp Thr Lys Lys
1175 1180 1185

Met Thr Ser Asn Phe Pro Val Asp Leu Ser Asp Tyr Pro Lys Ser
1190 1195 1200

Leu His Met Tyr Ala Asn Arg Leu Leu Asp His Arg Val Pro Glu
1205 1210 1215

Thr Asp Met Thr Phe Arg His Val Gly Ser Lys Leu Ile Val Ala
1220 1225 1230

Met Ser Ser Trp Leu Gln Lys Ala Ser Gly Ser Leu Pro Tyr Thr
1235 1240 1245

Gln Thr Leu Gln Asp His Leu Asn Ser Leu Lys Glu Phe Asn Leu
1250 1255 1260

Gln Asn Met Gly Leu Pro Asp Phe His Ile Pro Glu Asn Leu Phe
1265 1270 1275

Leu Lys Ser Asp Gly Arg Val Lys Tyr Thr Leu Asn Lys Asn Ser
1280 1285 1290

Leu Lys Ile Glu Ile Pro Leu Pro Phe Gly Gly Lys Ser Ser Arg
1295 1300 1305

Asp Leu Lys Met Leu Glu Thr Val Arg Thr Pro Ala Leu His Phe
1310 1315 1320

Lys Ser Val Gly Phe His Leu Pro Ser Arg Glu Phe Gln Val Pro
1325 1330 1335

Thr Phe Thr Ile Pro Lys Leu Tyr Gln Leu Gln Val Pro Leu Leu

1340 1345 1350

Gly Val Leu Asp Leu Ser Thr Asn Val Tyr Ser Asn Leu Tyr Asn
1355 1360 1365

Trp Ser Ala Ser Tyr Ser Gly Gly Asn Thr Ser Thr Asp His Phe
1370 1375 1380

Ser Leu Arg Ala Arg Tyr His Met Lys Ala Asp Ser Val Val Asp
1385 1390 1395

Leu Leu Ser Tyr Asn Val Gln Gly Ser Gly Glu Thr Thr Tyr Asp
1400 1405 1410

His Lys Asn Thr Phe Thr Leu Ser Cys Asp Gly Ser Leu Arg His
1415 1420 1425

Lys Phe Leu Asp Ser Asn Ile Lys Phe Ser His Val Glu Lys Leu
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Gly Asn Asn Pro Val Ser Lys Gly Leu Leu Ile Phe Asp Ala Ser
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Ser Ser Trp Gly Pro Gln Met Ser Ala Ser Val His Leu Asp Ser
1460 1465 1470

Lys Lys Lys Gln His Leu Phe Val Lys Glu Val Lys Ile Asp Gly
1475 1480 1485

Gln Phe Arg Val Ser Ser Phe Tyr Ala Lys Gly Thr Tyr Gly Leu
1490 1495 1500

Ser Cys Gln Arg Asp Pro Asn Thr Gly Arg Leu Asn Gly Glu Ser
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Asn Leu Arg Phe Asn Ser Ser Tyr Leu Gln Gly Thr Asn Gln Ile
1520 1525 1530

Thr Gly Arg Tyr Glu Asp Gly Thr Leu Ser Leu Thr Ser Thr Ser
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Asp Leu Gln Ser Gly Ile Ile Lys Asn Thr Ala Ser Leu Lys Tyr
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Glu Asn Tyr Glu Leu Thr Leu Lys Ser Asp Thr Asn Gly Lys Tyr
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Lys Asn Phe Ala Thr Ser Asn Lys Met Asp Met Thr Phe Ser Lys
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Gln Asn Ala Leu Leu Arg Ser Glu Tyr Gln Ala Asp Tyr Glu Ser
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Leu Arg Phe Phe Ser Leu Leu Ser Gly Ser Leu Asn Ser His Gly
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Leu Glu Leu Asn Ala Asp Ile Leu Gly Thr Asp Lys Ile Asn Ser
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Gly Ala His Lys Ala Thr Leu Arg Ile Gly Gln Asp Gly Ile Ser
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Thr Ser Ala Thr Thr Asn Leu Lys Cys Ser Leu Leu Val Leu Glu
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Asn Glu Leu Asn Ala Glu Leu Gly Leu Ser Gly Ala Ser Met Lys
1670 1675 1680

Leu Thr Thr Asn Gly Arg Phe Arg Glu His Asn Ala Lys Phe Ser
1685 1690 1695

Leu Asp Gly Lys Ala Ala Leu Thr Glu Leu Ser Leu Gly Ser Ala
1700 1705 1710

Tyr Gln Ala Met Ile Leu Gly Val Asp Ser Lys Asn Ile Phe Asn
1715 1720 1725

Phe Lys Val Ser Gln Glu Gly Leu Lys Leu Ser Asn Asp Met Met
1730 1735 1740

Gly Ser Tyr Ala Glu Met Lys Phe Asp His Thr Asn Ser Leu Asn
1745 1750 1755

Ile Ala Gly Leu Ser Leu Asp Phe Ser Ser Lys Leu Asp Asn Ile
1760 1765 1770

Tyr Ser Ser Asp Lys Phe Tyr Lys Gln Thr Val Asn Leu Gln Leu
1775 1780 1785

Gln Pro Tyr Ser Leu Val Thr Thr Leu Asn Ser Asp Leu Lys Tyr
1790 1795 1800

Asn Ala Leu Asp Leu Thr Asn Asn Gly Lys Leu Arg Leu Glu Pro
1805 1810 1815

Leu Lys Leu His Val Ala Gly Asn Leu Lys Gly Ala Tyr Gln Asn
1820 1825 1830

Asn Glu Ile Lys His Ile Tyr Ala Ile Ser Ser Ala Ala Leu Ser
1835 1840 1845

Ala Ser Tyr Lys Ala Asp Thr Val Ala Lys Val Gln Gly Val Glu
1850 1855 1860

Phe Ser His Arg Leu Asn Thr Asp Ile Ala Gly Leu Ala Ser Ala
1865 1870 1875

Ile Asp Met Ser Thr Asn Tyr Asn Ser Asp Ser Leu His Phe Ser
1880 1885 1890

Asn Val Phe Arg Ser Val Met Ala Pro Phe Thr Met Thr Ile Asp
1895 1900 1905

Ala His Thr Asn Gly Asn Gly Lys Leu Ala Leu Trp Gly Glu His
1910 1915 1920

Thr Gly Gln Leu Tyr Ser Lys Phe Leu Leu Lys Ala Glu Pro Leu
1925 1930 1935

Ala Phe Thr Phe Ser His Asp Tyr Lys Gly Ser Thr Ser His His
1940 1945 1950

Leu Val Ser Arg Lys Ser Ile Ser Ala Ala Leu Glu His Lys Val
1955 1960 1965

Ser Ala Leu Leu Thr Pro Ala Glu Gln Thr Gly Thr Trp Lys Leu
1970 1975 1980

Lys Thr Gln Phe Asn Asn Asn Glu Tyr Ser Gln Asp Leu Asp Ala
1985 1990 1995

Tyr Asn Thr Lys Asp Lys Ile Gly Val Glu Leu Thr Gly Arg Thr
2000 2005 2010

Leu Ala Asp Leu Thr Leu Leu Asp Ser Pro Ile Lys Val Pro Leu
2015 2020 2025

Leu Leu Ser Glu Pro Ile Asn Ile Ile Asp Ala Leu Glu Met Arg
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Asp Ala Val Glu Lys Pro Gln Glu Phe Thr Ile Val Ala Phe Val
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Lys Tyr Asp Lys Asn Gln Asp Val His Ser Ile Asn Leu Pro Phe
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Phe Glu Thr Leu Gln Glu Tyr Phe Glu Arg Asn Arg Gln Thr Ile
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Ile Val Val Val Glu Asn Val Gln Arg Asn Leu Lys His Ile Asn
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Pro Gln Gln Ala Asn Asp Tyr Leu Asn Ser Phe Asn Trp Glu Arg
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Gln Val Ser His Ala Lys Glu Lys Leu Thr Ala Leu Thr Lys Lys
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Tyr Arg Ile Thr Glu Asn Asp Ile Gln Ile Ala Leu Asp Asp Ala
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Lys Ile Asn Phe Asn Glu Lys Leu Ser Gln Leu Gln Thr Tyr Met
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Ile Gln Phe Asp Gln Tyr Ile Lys Asp Ser Tyr Asp Leu His Asp
2180 2185 2190

Leu Lys Ile Ala Ile Ala Asn Ile Ile Asp Glu Ile Ile Glu Lys
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Leu Lys Ser Leu Asp Glu His Tyr His Ile Arg Val Asn Leu Val
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Lys Thr Ile His Asp Leu His Leu Phe Ile Glu Asn Ile Asp Phe
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Asn Lys Ser Gly Ser Ser Thr Ala Ser Trp Ile Gln Asn Val Asp
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Thr Lys Tyr Gln Ile Arg Ile Gln Ile Gln Glu Lys Leu Gln Gln
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Leu Lys Arg His Ile Gln Asn Ile Asp Ile Gln His Leu Ala Gly
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Lys Leu Lys Gln His Ile Glu Ala Ile Asp Val Arg Val Leu Leu
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Asp Gln Leu Gly Thr Thr Ile Ser Phe Glu Arg Ile Asn Asp Val
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Leu Glu His Val Lys His Phe Val Ile Asn Leu Ile Gly Asp Phe
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Glu Val Ala Glu Lys Ile Asn Ala Phe Arg Ala Lys Val His Glu
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Leu Ile Glu Arg Tyr Glu Val Asp Gln Gln Ile Gln Val Leu Met
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Asp Lys Leu Val Glu Leu Thr His Gln Tyr Lys Leu Lys Glu Thr
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Ile Gln Lys Leu Ser Asn Val Leu Gln Gln Val Lys Ile Lys Asp
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Tyr Phe Glu Lys Leu Val Gly Phe Ile Asp Asp Ala Val Lys Lys
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Leu Asn Glu Leu Ser Phe Lys Thr Phe Ile Glu Asp Val Asn Lys
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Phe Leu Asp Met Leu Ile Lys Lys Leu Lys Ser Phe Asp Tyr His
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Gln Phe Val Asp Glu Thr Asn Asp Lys Ile Arg Glu Val Thr Gln
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Arg Leu Asn Gly Glu Ile Gln Ala Leu Glu Leu Pro Gln Lys Ala
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Glu Ala Leu Lys Leu Phe Leu Glu Glu Thr Lys Ala Thr Val Ala
2465 2470 2475

Val Tyr Leu Glu Ser Leu Gln Asp Thr Lys Ile Thr Leu Ile Ile
2480 2485 2490

Asn Trp Leu Gln Glu Ala Leu Ser Ser Ala Ser Leu Ala His Met

2495 2500 2505

Lys Ala Lys Phe Arg Glu Thr Leu Glu Asp Thr Arg Asp Arg Met
2510 2515 2520

Tyr Gln Met Asp Ile Gln Gln Glu Leu Gln Arg Tyr Leu Ser Leu
2525 2530 2535

Val Gly Gln Val Tyr Ser Thr Leu Val Thr Tyr Ile Ser Asp Trp
2540 2545 2550

Trp Thr Leu Ala Ala Lys Asn Leu Thr Asp Phe Ala Glu Gln Tyr
2555 2560 2565

Ser Ile Gln Asp Trp Ala Lys Arg Met Lys Ala Leu Val Glu Gln
2570 2575 2580

Gly Phe Thr Val Pro Glu Ile Lys Thr Ile Leu Gly Thr Met Pro
2585 2590 2595

Ala Phe Glu Val Ser Leu Gln Ala Leu Gln Lys Ala Thr Phe Gln
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Thr Pro Asp Phe Ile Val Pro Leu Thr Asp Leu Arg Ile Pro Ser
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Val Gln Ile Asn Phe Lys Asp Leu Lys Asn Ile Lys Ile Pro Ser
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Arg Phe Ser Thr Pro Glu Phe Thr Ile Leu Asn Thr Phe His Ile
2645 2650 2655

Pro Ser Phe Thr Ile Asp Phe Val Glu Met Lys Val Lys Ile Ile
2660 2665 2670

Arg Thr Ile Asp Gln Met Gln Asn Ser Glu Leu Gln Trp Pro Val
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Pro Asp Ile Tyr Leu Arg Asp Leu Lys Val Glu Asp Ile Pro Leu
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Ala Arg Ile Thr Leu Pro Asp Phe Arg Leu Pro Glu Ile Ala Ile
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Pro Glu Phe Ile Ile Pro Thr Leu Asn Leu Asn Asp Phe Gln Val
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Pro Asp Leu His Ile Pro Glu Phe Gln Leu Pro His Ile Ser His
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Thr Ile Glu Val Pro Thr Phe Gly Lys Leu Tyr Ser Ile Leu Lys
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Ile Gln Ser Pro Leu Phe Thr Leu Asp Ala Asn Ala Asp Ile Gly
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Asn Gly Thr Thr Ser Ala Asn Glu Ala Gly Ile Ala Ala Ser Ile
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Thr Ala Lys Gly Glu Ser Lys Leu Glu Val Leu Asn Phe Asp Phe
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Gln Ala Asn Ala Gln Leu Ser Asn Pro Lys Ile Asn Pro Leu Ala
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Leu Lys Glu Ser Val Lys Phe Ser Ser Lys Tyr Leu Arg Thr Glu
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His Gly Ser Glu Met Leu Phe Phe Gly Asn Ala Ile Glu Gly Lys
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Ser Asn Thr Val Ala Ser Leu His Thr Glu Lys Asn Thr Leu Glu
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Leu Ser Asn Gly Val Ile Val Lys Ile Asn Asn Gln Leu Thr Leu
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Asp Ser Asn Thr Lys Tyr Phe His Lys Leu Asn Ile Pro Lys Leu
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Asp Phe Ser Ser Gln Ala Asp Leu Arg Asn Glu Ile Lys Thr Leu
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Leu Lys Ala Gly His Ile Ala Trp Thr Ser Ser Gly Lys Gly Ser
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Trp Lys Trp Ala Cys Pro Arg Phe Ser Asp Glu Gly Thr His Glu
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Ser Gln Ile Ser Phe Thr Ile Glu Gly Pro Leu Thr Ser Phe Gly
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Leu Ser Asn Lys Ile Asn Ser Lys His Leu Arg Val Asn Gln Asn
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Leu Val Tyr Glu Ser Gly Ser Leu Asn Phe Ser Lys Leu Glu Ile
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Gln Ser Gln Val Asp Ser Gln His Val Gly His Ser Val Leu Thr
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Ala Lys Gly Met Ala Leu Phe Gly Glu Gly Lys Ala Glu Phe Thr
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Gly Arg His Asp Ala His Leu Asn Gly Lys Val Ile Gly Thr Leu
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Lys Asn Ser Leu Phe Phe Ser Ala Gln Pro Phe Glu Ile Thr Ala
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Ser Thr Asn Asn Glu Gly Asn Leu Lys Val Arg Phe Pro Leu Arg
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Leu Thr Gly Lys Ile Asp Phe Leu Asn Asn Tyr Ala Leu Phe Leu
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Ser Pro Ser Ala Gln Gln Ala Ser Trp Gln Val Ser Ala Arg Phe
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Asn Gln Tyr Lys Tyr Asn Gln Asn Phe Ser Ala Gly Asn Asn Glu
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Asn Ile Met Glu Ala His Val Gly Ile Asn Gly Glu Ala Asn Leu
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Asp Phe Leu Asn Ile Pro Leu Thr Ile Pro Glu Met Arg Leu Pro
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Tyr Thr Ile Ile Thr Thr Pro Pro Leu Lys Asp Phe Ser Leu Trp
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Glu Lys Thr Gly Leu Lys Glu Phe Leu Lys Thr Thr Lys Gln Ser
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Phe Asp Leu Ser Val Lys Ala Gln Tyr Lys Lys Asn Lys His Arg
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His Ser Ile Thr Asn Pro Leu Ala Val Leu Cys Glu Phe Ile Ser
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Gln Ser Ile Lys Ser Phe Asp Arg His Phe Glu Lys Asn Arg Asn
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Asn Ala Leu Asp Phe Val Thr Lys Ser Tyr Asn Glu Thr Lys Ile
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Lys Phe Asp Lys Tyr Lys Ala Glu Lys Ser His Asp Glu Leu Pro
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Arg Thr Phe Gln Ile Pro Gly Tyr Thr Val Pro Val Val Asn Val
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Glu Val Ser Pro Phe Thr Ile Glu Met Ser Ala Phe Gly Tyr Val
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Phe Pro Lys Ala Val Ser Met Pro Ser Phe Ser Ile Leu Gly Ser
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Asp Val Arg Val Pro Ser Tyr Thr Leu Ile Leu Pro Ser Leu Glu
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Leu Pro Val Leu His Val Pro Arg Asn Leu Lys Leu Ser Leu Pro
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His Phe Lys Glu Leu Cys Thr Ile Ser His Ile Phe Ile Pro Ala
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Met Gly Asn Ile Thr Tyr Asp Phe Ser Phe Lys Ser Ser Val Ile
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Thr Leu Asn Thr Asn Ala Glu Leu Phe Asn Gln Ser Asp Ile Val
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Ala His Leu Leu Ser Ser Ser Ser Ser Val Ile Asp Ala Leu Gln
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Tyr Lys Leu Glu Gly Thr Thr Arg Leu Thr Arg Lys Arg Gly Leu
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Lys Leu Ala Thr Ala Leu Ser Leu Ser Asn Lys Phe Val Glu Gly
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Ser His Asn Ser Thr Val Ser Leu Thr Thr Lys Asn Met Glu Val
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Ser Val Ala Lys Thr Thr Lys Ala Glu Ile Pro Ile Leu Arg Met
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Asn Phe Lys Gln Glu Leu Asn Gly Asn Thr Lys Ser Lys Pro Thr
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Asp Val Lys Gly Ser Val Leu Ser Arg Glu Tyr Ser Gly Thr Ile
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Ser Val Lys Leu Gln Gly Thr Ser Lys Ile Asp Asp Ile Trp Asn
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Glu Gly Leu Phe Phe Thr Asn Gly Glu His Thr Ser Lys Ala Thr
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Leu Glu Leu Ser Pro Trp Gln Met Ser Ala Leu Val Gln Val His
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Ala Ser Gln Pro Ser Ser Phe His Asp Phe Pro Asp Leu Gly Gln
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Glu Val Ala Leu Asn Ala Asn Thr Lys Asn Gln Lys Ile Arg Trp
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Lys Asn Glu Val Arg Ile His Ser Gly Ser Phe Gln Ser Gln Val
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Glu Leu Ser Asn Asp Gln Glu Lys Ala His Leu Asp Ile Ala Gly

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Ser Leu Glu Gly His Leu Arg Phe Leu Lys Asn Ile Ile Leu Pro
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Val Tyr Asp Lys Ser Leu Trp Asp Phe Leu Lys Leu Asp Val Thr
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Thr Ser Ile Gly Arg Arg Gln His Leu Arg Val Ser Thr Ala Phe
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Val Tyr Thr Lys Asn Pro Asn Gly Tyr Ser Phe Ser Ile Pro Val
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Lys Val Leu Ala Asp Lys Phe Ile Thr Pro Gly Leu Lys Leu Asn
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Asp Leu Asn Ser Val Leu Val Met Pro Thr Phe His Val Pro Phe
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Thr Asp Leu Gln Val Pro Ser Cys Lys Leu Asp Phe Arg Glu Ile
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Gln Ile Tyr Lys Lys Leu Arg Thr Ser Ser Phe Ala Leu Asn Leu
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Pro Thr Leu Pro Glu Val Lys Phe Pro Glu Val Asp Val Leu Thr
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Thr Val Pro Glu Ser Gln Leu Thr Val Ser Gln Phe Thr Leu Pro
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Ala Asn Lys Ile Ala Asp Phe Glu Leu Pro Thr Ile Ile Val Pro
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Glu Gln Thr Ile Glu Ile Pro Ser Ile Lys Phe Ser Val Pro Ala
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Gly Ile Val Ile Pro Ser Phe Gln Ala Leu Thr Ala Arg Phe Glu
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Val Asp Ser Pro Val Tyr Asn Ala Thr Trp Ser Ala Ser Leu Lys
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Asn Lys Ala Asp Tyr Val Glu Thr Val Leu Asp Ser Thr Cys Ser
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Ser Thr Val Gln Phe Leu Glu Tyr Glu Leu Asn Val Leu Gly Thr
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His Lys Ile Glu Asp Gly Thr Leu Ala Ser Lys Thr Lys Gly Thr
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Leu Ala His Arg Asp Phe Ser Ala Glu Tyr Glu Glu Asp Gly Lys
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Lys Ser Pro Ala Phe Thr Asp Leu His Leu Arg Tyr Gln Lys Asp
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Lys Lys Gly Ile Ser Thr Ser Ala Ala Ser Pro Ala Val Gly Thr
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Val Gly Met Asp Met Asp Glu Asp Asp Asp Phe Ser Lys Trp Asn
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Phe Lys Thr Glu Leu Arg Val Arg Glu Ser Asp Glu Glu Thr Gln
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Glu Val Ser Ser Lys Leu Arg Arg Asn Leu Gln Asn Asn Ala Glu
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Arg Phe Gln Lys Ala Ala Ser Gly Thr Thr Gly Thr Tyr Gln Glu
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Trp Lys Asp Lys Ala Gln Asn Leu Tyr Gln Glu Leu Leu Thr Gln
4145 4150 4155

Glu Gly Gln Ala Ser Phe Gln Gly Leu Lys Asp Asn Val Phe Asp
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Gly Leu Val Arg Val Thr Gln Lys Phe His Met Lys Val Lys His
4175 4180 4185

Leu Ile Asp Ser Leu Ile Asp Phe Leu Asn Phe Pro Arg Phe Gln
4190 4195 4200

Phe Pro Gly Lys Pro Gly Ile Tyr Thr Arg Glu Glu Leu Cys Thr
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Met Phe Ile Arg Glu Val Gly Thr Val Leu Ser Gln Val Tyr Ser
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Lys Val His Asn Gly Ser Glu Ile Leu Phe Ser Tyr Phe Gln Asp
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Leu Val Ile Thr Leu Pro Phe Glu Leu Arg Lys His Lys Leu Ile
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Asp Val Ile Ser Met Tyr Arg Glu Leu Leu Lys Asp Leu Ser Lys
4265 4270 4275

Glu Ala Gln Glu Val Phe Lys Ala Ile Gln Ser Leu Lys Thr Thr
4280 4285 4290

Glu Val Leu Arg Asn Leu Gln Asp Leu Leu Gln Phe Ile Phe Gln
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Leu Ile Glu Asp Asn Ile Lys Gln Leu Lys Glu Met Lys Phe Thr
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Tyr Leu Ile Asn Tyr Ile Gln Asp Glu Ile Asn Thr Ile Phe Asn
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Asp Tyr Ile Pro Tyr Val Phe Lys Leu Leu Lys Glu Asn Leu Cys
4340 4345 4350

Leu Asn Leu His Lys Phe Asn Glu Phe Ile Gln Asn Glu Leu Gln
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Glu Ala Ser Gln Glu Leu Gln Gln Ile His Gln Tyr Ile Met Ala
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Leu Arg Glu Glu Tyr Phe Asp Pro Ser Ile Val Gly Trp Thr Val
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Lys Tyr Tyr Glu Leu Glu Glu Lys Ile Val Ser Leu Ile Lys Asn
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Leu Leu Val Ala Leu Lys Asp Phe His Ser Glu Tyr Ile Val Ser
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Ala Ser Asn Phe Thr Ser Gln Leu Ser Ser Gln Val Glu Gln Phe
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Leu His Arg Asn Ile Gln Glu Tyr Leu Ser Ile Leu Thr Asp Pro
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Asp Gly Lys Gly Lys Glu Lys Ile Ala Glu Leu Ser Ala Thr Ala
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Gln Glu Ile Ile Lys Ser Gln Ala Ile Ala Thr Lys Lys Ile Ile
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Ser Asp Tyr His Gln Gln Phe Arg Tyr Lys Leu Gln Asp Phe Ser
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Arg Leu Ile Asp Leu Ser Ile Gln Asn Tyr His Thr Phe Leu Ile
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Ala His Thr Val Val Thr Ser Arg Val Val Asn Arg Ala Asn Thr Val
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Gln Glu Ala Thr Phe Gln Met Glu Leu Pro Lys Lys Ala Phe Ile Thr
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Asn Phe Ser Met Asn Ile Asp Gly Met Thr Tyr Pro Gly Ile Ile Lys
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Glu Lys Ala Glu Ala Gln Ala Gln Tyr Ser Ala Ala Val Ala Lys Gly
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Lys Ser Ala Gly Leu Val Lys Ala Thr Gly Arg Asn Met Glu Gln Phe
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Gln Val Ser Val Ser Val Ala Pro Asn Ala Lys Ile Thr Phe Glu Leu
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Val Tyr Glu Glu Leu Leu Lys Arg Arg Leu Gly Val Tyr Glu Leu Leu
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Leu Lys Val Arg Pro Gln Gln Leu Val Lys His Leu Gln Met Asp Ile
165 170 175

His Ile Phe Glu Pro Gln Gly Ile Ser Phe Leu Glu Thr Glu Ser Thr
180 185 190

Phe Met Thr Asn Gln Leu Val Asp Ala Leu Thr Thr Trp Gln Asn Lys
195 200 205

Thr Lys Ala His Ile Arg Phe Lys Pro Thr Leu Ser Gln Gln Gln Lys

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Ser Pro Glu Gln Gln Glu Thr Val Leu Asp Gly Asn Leu Ile Ile Arg
225                 230                 235                 240

Tyr Asp Val Asp Arg Ala Ile Ser Gly Gly Ser Ile Gln Ile Glu Asn
                245                 250                 255

Gly Tyr Phe Val His Tyr Phe Ala Pro Glu Gly Leu Thr Thr Met Pro
            260                 265                 270

Lys Asn Val Val Phe Val Ile Asp Lys Ser Gly Ser Met Ser Gly Arg
        275                 280                 285

Lys Ile Gln Gln Thr Arg Glu Ala Leu Ile Lys Ile Leu Asp Asp Leu
    290                 295                 300

Ser Pro Arg Asp Gln Phe Asn Leu Ile Val Phe Ser Thr Glu Ala Thr
305                 310                 315                 320

Gln Trp Arg Pro Ser Leu Val Pro Ala Ser Ala Glu Asn Val Asn Lys
                325                 330                 335

Ala Arg Ser Phe Ala Ala Gly Ile Gln Ala Leu Gly Gly Thr Asn Ile
            340                 345                 350

Asn Asp Ala Met Leu Met Ala Val Gln Leu Leu Asp Ser Ser Asn Gln
        355                 360                 365

Glu Glu Arg Leu Pro Glu Gly Ser Val Ser Leu Ile Ile Leu Leu Thr
    370                 375                 380

Asp Gly Asp Pro Thr Val Gly Glu Thr Asn Pro Arg Ser Ile Gln Asn
385                 390                 395                 400

Asn Val Arg Glu Ala Val Ser Gly Arg Tyr Ser Leu Phe Cys Leu Gly
                405                 410                 415

Phe Gly Phe Asp Val Ser Tyr Ala Phe Leu Glu Lys Leu Ala Leu Asp
            420                 425                 430

Asn Gly Gly Leu Ala Arg Arg Ile His Glu Asp Ser Asp Ser Ala Leu
        435                 440                 445

Gln Leu Gln Asp Phe Tyr Gln Glu Val Ala Asn Pro Leu Leu Thr Ala
    450                 455                 460

Val Thr Phe Glu Tyr Pro Ser Asn Ala Val Glu Glu Val Thr Gln Asn
465                 470                 475                 480

Asn Phe Arg Leu Leu Phe Lys Gly Ser Glu Met Val Val Ala Gly Lys
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Leu Gln Asp Arg Gly Pro Asp Val Leu Thr Ala Thr Val Ser Gly Lys
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Leu Pro Thr Gln Asn Ile Thr Phe Gln Thr Glu Ser Ser Val Ala Glu
        515                 520                 525
```

```
Gln Glu Ala Glu Phe Gln Ser Pro Lys Tyr   Ile Phe His Asn Phe Met
    530                 535                   540

Glu Arg Leu Trp Ala Tyr Leu Thr Ile Gln Gln Leu Leu Glu Gln Thr
545                 550                 555                 560

Val Ser Ala Ser Asp Ala Asp Gln Gln Ala Leu Arg Asn Gln Ala Leu
                565                 570                 575

Asn Leu Ser Leu Ala Tyr Ser Phe Val Thr Pro Leu Thr Ser Met Val
            580                 585                 590

Val Thr Lys Pro Asp Asp Gln Glu Gln Ser Gln Val Ala Glu Lys Pro
        595                 600                 605

Met Glu Gly Glu Ser Arg Asn Arg Asn Val His Ser Gly Ser Thr Phe
    610                 615                 620

Phe Lys Tyr Tyr Leu Gln Gly Ala Lys Ile Pro Lys Pro Glu Ala Ser
625                 630                 635                 640

Phe Ser Pro Arg Arg Gly Trp Asn Arg Gln Ala Gly Ala Ala Gly Ser
                645                 650                 655

Arg Met Asn Phe Arg Pro Gly Val Leu Ser Ser Arg Gln Leu Gly Leu
            660                 665                 670

Pro Gly Pro Pro Asp Val Pro Asp His Ala Ala Tyr His Pro Phe Arg
        675                 680                 685

Arg Leu Ala Ile Leu Pro Ala Ser Ala Pro Pro Ala Thr Ser Asn Pro
    690                 695                 700

Asp Pro Ala Val Ser Arg Val Met Asn Met Lys Ile Glu Glu Thr Thr
705                 710                 715                 720

Met Thr Thr Gln Thr Pro Ala Pro Ile Gln Ala Pro Ser Ala Ile Leu
                725                 730                 735

Pro Leu Pro Gly Gln Ser Val Glu Arg Leu Cys Val Asp Pro Arg His
            740                 745                 750

Arg Gln Gly Pro Val Asn Leu Leu Ser Asp Pro Glu Gln Gly Val Glu
        755                 760                 765

Val Thr Gly Gln Tyr Glu Arg Glu Lys Ala Gly Phe Ser Trp Ile Glu
    770                 775                 780

Val Thr Phe Lys Asn Pro Leu Val Trp Val His Ala Ser Pro Glu His
785                 790                 795                 800

Val Val Val Thr Arg Asn Arg Arg Ser Ser Ala Tyr Lys Trp Lys Glu
                805                 810                 815

Thr Leu Phe Ser Val Met Pro Gly Leu Lys Met Thr Met Asp Lys Thr
            820                 825                 830
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Gly Leu Leu Leu Leu Ser Asp Pro Asp Lys Val Thr Ile Gly Leu Leu
835 840 845

Phe Trp Asp Gly Arg Gly Glu Gly Leu Arg Leu Leu Leu Arg Asp Thr
850 855 860

Asp Arg Phe Ser Ser His Val Gly Gly Thr Leu Gly Gln Phe Tyr Gln
865 870 875 880

Glu Val Leu Trp Gly Ser Pro Ala Ala Ser Asp Asp Gly Arg Arg Thr
885 890 895

Leu Arg Val Gln Gly Asn Asp His Ser Ala Thr Arg Glu Arg Arg Leu
900 905 910

Asp Tyr Gln Glu Gly Pro Pro Gly Val Glu Ile Ser Cys Trp Ser Val
915 920 925

Glu Leu
930

<210> 43
<211> 1744
<212> PRT
<213> Homo sapiens

<300>
<308> Swiss-Prot/P01028
<309> 1986-07-21
<313> (1)..(1744)

<400> 43

Met Arg Leu Leu Trp Gly Leu Ile Trp Ala Ser Ser Phe Phe Thr Leu
1 5 10 15

Ser Leu Gln Lys Pro Arg Leu Leu Leu Phe Ser Pro Ser Val Val His
20 25 30

Leu Gly Val Pro Leu Ser Val Gly Val Gln Leu Gln Asp Val Pro Arg
35 40 45

Gly Gln Val Val Lys Gly Ser Val Phe Leu Arg Asn Pro Ser Arg Asn
50 55 60

Asn Val Pro Cys Ser Pro Lys Val Asp Phe Thr Leu Ser Ser Glu Arg
65 70 75 80

Asp Phe Ala Leu Leu Ser Leu Gln Val Pro Leu Lys Asp Ala Lys Ser
85 90 95

Cys Gly Leu His Gln Leu Leu Arg Gly Pro Glu Val Gln Leu Val Ala
100 105 110

His Ser Pro Trp Leu Lys Asp Ser Leu Ser Arg Thr Thr Asn Ile Gln
115 120 125

Gly Ile Asn Leu Leu Phe Ser Ser Arg Arg Gly His Leu Phe Leu Gln
130 135 140

Thr Asp Gln Pro Ile Tyr Asn Pro Gly Gln Arg Val Arg Tyr Arg Val
145 150 155 160

Phe Ala Leu Asp Gln Lys Met Arg Pro Ser Thr Asp Thr Ile Thr Val
165 170 175

Met Val Glu Asn Ser His Gly Leu Arg Val Arg Lys Lys Glu Val Tyr
180 185 190

Met Pro Ser Ser Ile Phe Gln Asp Asp Phe Val Ile Pro Asp Ile Ser
195 200 205

Glu Pro Gly Thr Trp Lys Ile Ser Ala Arg Phe Ser Asp Gly Leu Glu
210 215 220

Ser Asn Ser Ser Thr Gln Phe Glu Val Lys Lys Tyr Val Leu Pro Asn
225 230 235 240

Phe Glu Val Lys Ile Thr Pro Gly Lys Pro Tyr Ile Leu Thr Val Pro
245 250 255

Gly His Leu Asp Glu Met Gln Leu Asp Ile Gln Ala Arg Tyr Ile Tyr
260 265 270

Gly Lys Pro Val Gln Gly Val Ala Tyr Val Arg Phe Gly Leu Leu Asp
275 280 285

Glu Asp Gly Lys Lys Thr Phe Phe Arg Gly Leu Glu Ser Gln Thr Lys
290 295 300

Leu Val Asn Gly Gln Ser His Ile Ser Leu Ser Lys Ala Glu Phe Gln
305 310 315 320

Asp Ala Leu Glu Lys Leu Asn Met Gly Ile Thr Asp Leu Gln Gly Leu
325 330 335

Arg Leu Tyr Val Ala Ala Ala Ile Ile Glu Ser Pro Gly Gly Glu Met
340 345 350

Glu Glu Ala Glu Leu Thr Ser Trp Tyr Phe Val Ser Ser Pro Phe Ser
355 360 365

Leu Asp Leu Ser Lys Thr Lys Arg His Leu Val Pro Gly Ala Pro Phe
370 375 380

Leu Leu Gln Ala Leu Val Arg Glu Met Ser Gly Ser Pro Ala Ser Gly
385 390 395 400

Ile Pro Val Lys Val Ser Ala Thr Val Ser Ser Pro Gly Ser Val Pro
405 410 415

Glu Val Gln Asp Ile Gln Gln Asn Thr Asp Gly Ser Gly Gln Val Ser
420 425 430

Ile Pro Ile Ile Ile Pro Gln Thr Ile Ser Glu Leu Gln Leu Ser Val
435 440 445

Ser Ala Gly Ser Pro His Pro Ala Ile Ala Arg Leu Thr Val Ala Ala

450 455 460

Pro Pro Ser Gly Gly Pro Gly Phe Leu Ser Ile Glu Arg Pro Asp Ser
465 470 475 480

Arg Pro Pro Arg Val Gly Asp Thr Leu Asn Leu Asn Leu Arg Ala Val
485 490 495

Gly Ser Gly Ala Thr Phe Ser His Tyr Tyr Tyr Met Ile Leu Ser Arg
500 505 510

Gly Gln Ile Val Phe Met Asn Arg Glu Pro Lys Arg Thr Leu Thr Ser
515 520 525

Val Ser Val Phe Val Asp His His Leu Ala Pro Ser Phe Tyr Phe Val
530 535 540

Ala Phe Tyr Tyr His Gly Asp His Pro Val Ala Asn Ser Leu Arg Val
545 550 555 560

Asp Val Gln Ala Gly Ala Cys Glu Gly Lys Leu Glu Leu Ser Val Asp
565 570 575

Gly Ala Lys Gln Tyr Arg Asn Gly Glu Ser Val Lys Leu His Leu Glu
580 585 590

Thr Asp Ser Leu Ala Leu Val Ala Leu Gly Ala Leu Asp Thr Ala Leu
595 600 605

Tyr Ala Ala Gly Ser Lys Ser His Lys Pro Leu Asn Met Gly Lys Val
610 615 620

Phe Glu Ala Met Asn Ser Tyr Asp Leu Gly Cys Gly Pro Gly Gly Gly
625 630 635 640

Asp Ser Ala Leu Gln Val Phe Gln Ala Ala Gly Leu Ala Phe Ser Asp
645 650 655

Gly Asp Gln Trp Thr Leu Ser Arg Lys Arg Leu Ser Cys Pro Lys Glu
660 665 670

Lys Thr Thr Arg Lys Lys Arg Asn Val Asn Phe Gln Lys Ala Ile Asn
675 680 685

Glu Lys Leu Gly Gln Tyr Ala Ser Pro Thr Ala Lys Arg Cys Cys Gln
690 695 700

Asp Gly Val Thr Arg Leu Pro Met Met Arg Ser Cys Glu Gln Arg Ala
705 710 715 720

Ala Arg Val Gln Gln Pro Asp Cys Arg Glu Pro Phe Leu Ser Cys Cys
725 730 735

Gln Phe Ala Glu Ser Leu Arg Lys Lys Ser Arg Asp Lys Gly Gln Ala
740 745 750

Gly Leu Gln Arg Ala Leu Glu Ile Leu Gln Glu Glu Asp Leu Ile Asp
755 760 765

Glu Asp Asp Ile Pro Val Arg Ser Phe Phe Pro Glu Asn Trp Leu Trp
770 775 780

Arg Val Glu Thr Val Asp Arg Phe Gln Ile Leu Thr Leu Trp Leu Pro
785 790 795 800

Asp Ser Leu Thr Thr Trp Glu Ile His Gly Leu Ser Leu Ser Lys Thr
805 810 815

Lys Gly Leu Cys Val Ala Thr Pro Val Gln Leu Arg Val Phe Arg Glu
820 825 830

Phe His Leu His Leu Arg Leu Pro Met Ser Val Arg Arg Phe Glu Gln
835 840 845

Leu Glu Leu Arg Pro Val Leu Tyr Asn Tyr Leu Asp Lys Asn Leu Thr
850 855 860

Val Ser Val His Val Ser Pro Val Glu Gly Leu Cys Leu Ala Gly Gly
865 870 875 880

Gly Gly Leu Ala Gln Gln Val Leu Val Pro Ala Gly Ser Ala Arg Pro
885 890 895

Val Ala Phe Ser Val Val Pro Thr Ala Ala Ala Ala Val Ser Leu Lys
900 905 910

Val Val Ala Arg Gly Ser Phe Glu Phe Pro Val Gly Asp Ala Val Ser
915 920 925

Lys Val Leu Gln Ile Glu Lys Glu Gly Ala Ile His Arg Glu Glu Leu
930 935 940

Val Tyr Glu Leu Asn Pro Leu Asp His Arg Gly Arg Thr Leu Glu Ile
945 950 955 960

Pro Gly Asn Ser Asp Pro Asn Met Ile Pro Asp Gly Asp Phe Asn Ser
965 970 975

Tyr Val Arg Val Thr Ala Ser Asp Pro Leu Asp Thr Leu Gly Ser Glu
980 985 990

Gly Ala Leu Ser Pro Gly Gly Val Ala Ser Leu Leu Arg Leu Pro Arg
995 1000 1005

Gly Cys Gly Glu Gln Thr Met Ile Tyr Leu Ala Pro Thr Leu Ala
1010 1015 1020

Ala Ser Arg Tyr Leu Asp Lys Thr Glu Gln Trp Ser Thr Leu Pro
1025 1030 1035

Pro Glu Thr Lys Asp His Ala Val Asp Leu Ile Gln Lys Gly Tyr
1040 1045 1050

Met Arg Ile Gln Gln Phe Arg Lys Ala Asp Gly Ser Tyr Ala Ala
1055 1060 1065

Trp Leu Ser Arg Asp Ser Ser Thr Trp Leu Thr Ala Phe Val Leu
1070 1075 1080

Lys Val Leu Ser Leu Ala Gln Glu Gln Val Gly Gly Ser Pro Glu
1085 1090 1095

Lys Leu Gln Glu Thr Ser Asn Trp Leu Leu Ser Gln Gln Gln Ala
1100 1105 1110

Asp Gly Ser Phe Gln Asp Pro Cys Pro Val Leu Asp Arg Ser Met
1115 1120 1125

Gln Gly Gly Leu Val Gly Asn Asp Glu Thr Val Ala Leu Thr Ala
1130 1135 1140

Phe Val Thr Ile Ala Leu His His Gly Leu Ala Val Phe Gln Asp
1145 1150 1155

Glu Gly Ala Glu Pro Leu Lys Gln Arg Val Glu Ala Ser Ile Ser
1160 1165 1170

Lys Ala Asn Ser Phe Leu Gly Glu Lys Ala Ser Ala Gly Leu Leu
1175 1180 1185

Gly Ala His Ala Ala Ala Ile Thr Ala Tyr Ala Leu Ser Leu Thr
1190 1195 1200

Lys Ala Pro Val Asp Leu Leu Gly Val Ala His Asn Asn Leu Met
1205 1210 1215

Ala Met Ala Gln Glu Thr Gly Asp Asn Leu Tyr Trp Gly Ser Val
1220 1225 1230

Thr Gly Ser Gln Ser Asn Ala Val Ser Pro Thr Pro Ala Pro Arg
1235 1240 1245

Asn Pro Ser Asp Pro Met Pro Gln Ala Pro Ala Leu Trp Ile Glu
1250 1255 1260

Thr Thr Ala Tyr Ala Leu Leu His Leu Leu Leu His Glu Gly Lys
1265 1270 1275

Ala Glu Met Ala Asp Gln Ala Ser Ala Trp Leu Thr Arg Gln Gly
1280 1285 1290

Ser Phe Gln Gly Gly Phe Arg Ser Thr Gln Asp Thr Val Ile Ala
1295 1300 1305

Leu Asp Ala Leu Ser Ala Tyr Trp Ile Ala Ser His Thr Thr Glu
1310 1315 1320

Glu Arg Gly Leu Asn Val Thr Leu Ser Ser Thr Gly Arg Asn Gly
1325 1330 1335

Phe Lys Ser His Ala Leu Gln Leu Asn Asn Arg Gln Ile Arg Gly
1340 1345 1350

Leu Glu Glu Glu Leu Gln Phe Ser Leu Gly Ser Lys Ile Asn Val
1355 1360 1365

Lys Val Gly Gly Asn Ser Lys Gly Thr Leu Lys Val Leu Arg Thr
1370 1375 1380

Tyr Asn Val Leu Asp Met Lys Asn Thr Thr Cys Gln Asp Leu Gln
1385 1390 1395

Ile Glu Val Thr Val Lys Gly His Val Glu Tyr Thr Met Glu Ala
1400 1405 1410

Asn Glu Asp Tyr Glu Asp Tyr Glu Tyr Asp Glu Leu Pro Ala Lys
1415 1420 1425

Asp Asp Pro Asp Ala Pro Leu Gln Pro Val Thr Pro Leu Gln Leu
1430 1435 1440

Phe Glu Gly Arg Arg Asn Arg Arg Arg Arg Glu Ala Pro Lys Val
1445 1450 1455

Val Glu Glu Gln Glu Ser Arg Val His Tyr Thr Val Cys Ile Trp
1460 1465 1470

Arg Asn Gly Lys Val Gly Leu Ser Gly Met Ala Ile Ala Asp Val
1475 1480 1485

Thr Leu Leu Ser Gly Phe His Ala Leu Arg Ala Asp Leu Glu Lys
1490 1495 1500

Leu Thr Ser Leu Ser Asp Arg Tyr Val Ser His Phe Glu Thr Glu
1505 1510 1515

Gly Pro His Val Leu Leu Tyr Phe Asp Ser Val Pro Thr Ser Arg
1520 1525 1530

Glu Cys Val Gly Phe Glu Ala Val Gln Glu Val Pro Val Gly Leu
1535 1540 1545

Val Gln Pro Ala Ser Ala Thr Leu Tyr Asp Tyr Tyr Asn Pro Glu
1550 1555 1560

Arg Arg Cys Ser Val Phe Tyr Gly Ala Pro Ser Lys Ser Arg Leu
1565 1570 1575

Leu Ala Thr Leu Cys Ser Ala Glu Val Cys Gln Cys Ala Glu Gly
1580 1585 1590

Lys Cys Pro Arg Gln Arg Arg Ala Leu Glu Arg Gly Leu Gln Asp
1595 1600 1605

Glu Asp Gly Tyr Arg Met Lys Phe Ala Cys Tyr Tyr Pro Arg Val
1610 1615 1620

Glu Tyr Gly Phe Gln Val Lys Val Leu Arg Glu Asp Ser Arg Ala
1625 1630 1635

Ala Phe Arg Leu Phe Glu Thr Lys Ile Thr Gln Val Leu His Phe

1640 1645 1650

Thr Lys Asp Val Lys Ala Ala Ala Asn Gln Met Arg Asn Phe Leu
1655 1660 1665

Val Arg Ala Ser Cys Arg Leu Arg Leu Glu Pro Gly Lys Glu Tyr
1670 1675 1680

Leu Ile Met Gly Leu Asp Gly Ala Thr Tyr Asp Leu Glu Gly His
1685 1690 1695

Pro Gln Tyr Leu Leu Asp Ser Asn Ser Trp Ile Glu Glu Met Pro
1700 1705 1710

Ser Glu Arg Leu Cys Arg Ser Thr Arg Gln Arg Ala Ala Cys Ala
1715 1720 1725

Gln Leu Asn Asp Phe Leu Gln Glu Tyr Gly Thr Gln Gly Cys Gln
1730 1735 1740

Val

<210> 44
<211> 1663
<212> PRT
<213> Homo sapiens

<300>
<308> Swiss-Prot/P01024
<309> 1986-07-21
<313> (1)..(1663)

<400> 44

Met Gly Pro Thr Ser Gly Pro Ser Leu Leu Leu Leu Leu Leu Thr His
1 5 10 15

Leu Pro Leu Ala Leu Gly Ser Pro Met Tyr Ser Ile Ile Thr Pro Asn
20 25 30

Ile Leu Arg Leu Glu Ser Glu Glu Thr Met Val Leu Glu Ala His Asp
35 40 45

Ala Gln Gly Asp Val Pro Val Thr Val Thr Val His Asp Phe Pro Gly
50 55 60

Lys Lys Leu Val Leu Ser Ser Glu Lys Thr Val Leu Thr Pro Ala Thr
65 70 75 80

Asn His Met Gly Asn Val Thr Phe Thr Ile Pro Ala Asn Arg Glu Phe
85 90 95

Lys Ser Glu Lys Gly Arg Asn Lys Phe Val Thr Val Gln Ala Thr Phe
100 105 110

Gly Thr Gln Val Val Glu Lys Val Val Leu Val Ser Leu Gln Ser Gly
115 120 125

Tyr Leu Phe Ile Gln Thr Asp Lys Thr Ile Tyr Thr Pro Gly Ser Thr
130 135 140

Val Leu Tyr Arg Ile Phe Thr Val Asn His Lys Leu Leu Pro Val Gly
145 150 155 160

Arg Thr Val Met Val Asn Ile Glu Asn Pro Glu Gly Ile Pro Val Lys
165 170 175

Gln Asp Ser Leu Ser Ser Gln Asn Gln Leu Gly Val Leu Pro Leu Ser
180 185 190

Trp Asp Ile Pro Glu Leu Val Asn Met Gly Gln Trp Lys Ile Arg Ala
195 200 205

Tyr Tyr Glu Asn Ser Pro Gln Gln Val Phe Ser Thr Glu Phe Glu Val
210 215 220

Lys Glu Tyr Val Leu Pro Ser Phe Glu Val Ile Val Glu Pro Thr Glu
225 230 235 240

Lys Phe Tyr Tyr Ile Tyr Asn Glu Lys Gly Leu Glu Val Thr Ile Thr
245 250 255

Ala Arg Phe Leu Tyr Gly Lys Lys Val Glu Gly Thr Ala Phe Val Ile
260 265 270

Phe Gly Ile Gln Asp Gly Glu Gln Arg Ile Ser Leu Pro Glu Ser Leu
275 280 285

Lys Arg Ile Pro Ile Glu Asp Gly Ser Gly Glu Val Val Leu Ser Arg
290 295 300

Lys Val Leu Leu Asp Gly Val Gln Asn Leu Arg Ala Glu Asp Leu Val
305 310 315 320

Gly Lys Ser Leu Tyr Val Ser Ala Thr Val Ile Leu His Ser Gly Ser
325 330 335

Asp Met Val Gln Ala Glu Arg Ser Gly Ile Pro Ile Val Thr Ser Pro
340 345 350

Tyr Gln Ile His Phe Thr Lys Thr Pro Lys Tyr Phe Lys Pro Gly Met
355 360 365

Pro Phe Asp Leu Met Val Phe Val Thr Asn Pro Asp Gly Ser Pro Ala
370 375 380

Tyr Arg Val Pro Val Ala Val Gln Gly Glu Asp Thr Val Gln Ser Leu
385 390 395 400

Thr Gln Gly Asp Gly Val Ala Lys Leu Ser Ile Asn Thr His Pro Ser
405 410 415

Gln Lys Pro Leu Ser Ile Thr Val Arg Thr Lys Lys Gln Glu Leu Ser
420 425 430

Glu Ala Glu Gln Ala Thr Arg Thr Met Gln Ala Leu Pro Tyr Ser Thr
435 440 445

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Val Gly Asn Ser Asn Asn Tyr Leu His Leu   Ser Val Leu Arg Thr Glu
    450                 455               460

Leu Arg Pro Gly Glu Thr Leu Asn Val Asn Phe Leu Leu Arg Met Asp
465                 470                 475                 480

Arg Ala His Glu Ala Lys Ile Arg Tyr Tyr Thr Tyr Leu Ile Met Asn
                485                 490                 495

Lys Gly Arg Leu Leu Lys Ala Gly Arg Gln Val Arg Glu Pro Gly Gln
            500                 505                 510

Asp Leu Val Val Leu Pro Leu Ser Ile Thr Thr Asp Phe Ile Pro Ser
        515                 520                 525

Phe Arg Leu Val Ala Tyr Tyr Thr Leu Ile Gly Ala Ser Gly Gln Arg
    530                 535                 540

Glu Val Val Ala Asp Ser Val Trp Val Asp Val Lys Asp Ser Cys Val
545                 550                 555                 560

Gly Ser Leu Val Val Lys Ser Gly Gln Ser Glu Asp Arg Gln Pro Val
                565                 570                 575

Pro Gly Gln Gln Met Thr Leu Lys Ile Glu Gly Asp His Gly Ala Arg
            580                 585                 590

Val Val Leu Val Ala Val Asp Lys Gly Val Phe Val Leu Asn Lys Lys
        595                 600                 605

Asn Lys Leu Thr Gln Ser Lys Ile Trp Asp Val Val Glu Lys Ala Asp
    610                 615                 620

Ile Gly Cys Thr Pro Gly Ser Gly Lys Asp Tyr Ala Gly Val Phe Ser
625                 630                 635                 640

Asp Ala Gly Leu Thr Phe Thr Ser Ser Ser Gly Gln Gln Thr Ala Gln
                645                 650                 655

Arg Ala Glu Leu Gln Cys Pro Gln Pro Ala Ala Arg Arg Arg Arg Ser
            660                 665                 670

Val Gln Leu Thr Glu Lys Arg Met Asp Lys Val Gly Lys Tyr Pro Lys
        675                 680                 685

Glu Leu Arg Lys Cys Cys Glu Asp Gly Met Arg Glu Asn Pro Met Arg
    690                 695                 700

Phe Ser Cys Gln Arg Arg Thr Arg Phe Ile Ser Leu Gly Glu Ala Cys
705                 710                 715                 720

Lys Lys Val Phe Leu Asp Cys Cys Asn Tyr Ile Thr Glu Leu Arg Arg
                725                 730                 735

Gln His Ala Arg Ala Ser His Leu Gly Leu Ala Arg Ser Asn Leu Asp
            740                 745                 750
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Glu Asp Ile Ile Ala Glu Glu Asn Ile Val   Ser Arg Ser Glu Phe Pro
        755                 760           765

Glu Ser Trp Leu Trp Asn Val Glu Asp Leu Lys Glu Pro Pro Lys Asn
    770                 775                 780

Gly Ile Ser Thr Lys Leu Met Asn Ile Phe Leu Lys Asp Ser Ile Thr
785                 790                 795                 800

Thr Trp Glu Ile Leu Ala Val Ser Met Ser Asp Lys Lys Gly Ile Cys
                805                 810                 815

Val Ala Asp Pro Phe Glu Val Thr Val Met Gln Asp Phe Phe Ile Asp
            820                 825                 830

Leu Arg Leu Pro Tyr Ser Val Val Arg Asn Glu Gln Val Glu Ile Arg
        835                 840                 845

Ala Val Leu Tyr Asn Tyr Arg Gln Asn Gln Glu Leu Lys Val Arg Val
    850                 855                 860

Glu Leu Leu His Asn Pro Ala Phe Cys Ser Leu Ala Thr Thr Lys Arg
865                 870                 875                 880

Arg His Gln Gln Thr Val Thr Ile Pro Pro Lys Ser Ser Leu Ser Val
                885                 890                 895

Pro Tyr Val Ile Val Pro Leu Lys Thr Gly Leu Gln Glu Val Glu Val
            900                 905                 910

Lys Ala Ala Val Tyr His His Phe Ile Ser Asp Gly Val Arg Lys Ser
        915                 920                 925

Leu Lys Val Val Pro Glu Gly Ile Arg Met Asn Lys Thr Val Ala Val
    930                 935                 940

Arg Thr Leu Asp Pro Glu Arg Leu Gly Arg Glu Gly Val Gln Lys Glu
945                 950                 955                 960

Asp Ile Pro Pro Ala Asp Leu Ser Asp Gln Val Pro Asp Thr Glu Ser
                965                 970                 975

Glu Thr Arg Ile Leu Leu Gln Gly Thr Pro Val Ala Gln Met Thr Glu
            980                 985                 990

Asp Ala Val Asp Ala Glu Arg Leu  Lys His Leu Ile Val  Thr Pro Ser
        995                 1000                1005

Gly Cys  Gly Glu Gln Asn Met  Ile Gly Met Thr Pro  Thr Val Ile
    1010                1015                1020

Ala Val  His Tyr Leu Asp Glu  Thr Glu Gln Trp Glu  Lys Phe Gly
    1025                1030                1035

Leu Glu  Lys Arg Gln Gly Ala  Leu Glu Leu Ile Lys  Lys Gly Tyr
    1040                1045                1050

Thr Gln  Gln Leu Ala Phe Arg  Gln Pro Ser Ser Ala  Phe Ala Ala
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1055 1060 1065

Phe Val Lys Arg Ala Pro Ser Thr Trp Leu Thr Ala Tyr Val Val
1070 1075 1080

Lys Val Phe Ser Leu Ala Val Asn Leu Ile Ala Ile Asp Ser Gln
1085 1090 1095

Val Leu Cys Gly Ala Val Lys Trp Leu Ile Leu Glu Lys Gln Lys
1100 1105 1110

Pro Asp Gly Val Phe Gln Glu Asp Ala Pro Val Ile His Gln Glu
1115 1120 1125

Met Ile Gly Gly Leu Arg Asn Asn Asn Glu Lys Asp Met Ala Leu
1130 1135 1140

Thr Ala Phe Val Leu Ile Ser Leu Gln Glu Ala Lys Asp Ile Cys
1145 1150 1155

Glu Glu Gln Val Asn Ser Leu Pro Gly Ser Ile Thr Lys Ala Gly
1160 1165 1170

Asp Phe Leu Glu Ala Asn Tyr Met Asn Leu Gln Arg Ser Tyr Thr
1175 1180 1185

Val Ala Ile Ala Gly Tyr Ala Leu Ala Gln Met Gly Arg Leu Lys
1190 1195 1200

Gly Pro Leu Leu Asn Lys Phe Leu Thr Thr Ala Lys Asp Lys Asn
1205 1210 1215

Arg Trp Glu Asp Pro Gly Lys Gln Leu Tyr Asn Val Glu Ala Thr
1220 1225 1230

Ser Tyr Ala Leu Leu Ala Leu Leu Gln Leu Lys Asp Phe Asp Phe
1235 1240 1245

Val Pro Pro Val Val Arg Trp Leu Asn Glu Gln Arg Tyr Tyr Gly
1250 1255 1260

Gly Gly Tyr Gly Ser Thr Gln Ala Thr Phe Met Val Phe Gln Ala
1265 1270 1275

Leu Ala Gln Tyr Gln Lys Asp Ala Pro Asp His Gln Glu Leu Asn
1280 1285 1290

Leu Asp Val Ser Leu Gln Leu Pro Ser Arg Ser Ser Lys Ile Thr
1295 1300 1305

His Arg Ile His Trp Glu Ser Ala Ser Leu Leu Arg Ser Glu Glu
1310 1315 1320

Thr Lys Glu Asn Glu Gly Phe Thr Val Thr Ala Glu Gly Lys Gly
1325 1330 1335

Gln Gly Thr Leu Ser Val Val Thr Met Tyr His Ala Lys Ala Lys
1340 1345 1350

Asp Gln Leu Thr Cys Asn Lys Phe Asp Leu Lys Val Thr Ile Lys
1355 1360 1365

Pro Ala Pro Glu Thr Glu Lys Arg Pro Gln Asp Ala Lys Asn Thr
1370 1375 1380

Met Ile Leu Glu Ile Cys Thr Arg Tyr Arg Gly Asp Gln Asp Ala
1385 1390 1395

Thr Met Ser Ile Leu Asp Ile Ser Met Met Thr Gly Phe Ala Pro
1400 1405 1410

Asp Thr Asp Asp Leu Lys Gln Leu Ala Asn Gly Val Asp Arg Tyr
1415 1420 1425

Ile Ser Lys Tyr Glu Leu Asp Lys Ala Phe Ser Asp Arg Asn Thr
1430 1435 1440

Leu Ile Ile Tyr Leu Asp Lys Val Ser His Ser Glu Asp Asp Cys
1445 1450 1455

Leu Ala Phe Lys Val His Gln Tyr Phe Asn Val Glu Leu Ile Gln
1460 1465 1470

Pro Gly Ala Val Lys Val Tyr Ala Tyr Tyr Asn Leu Glu Glu Ser
1475 1480 1485

Cys Thr Arg Phe Tyr His Pro Glu Lys Glu Asp Gly Lys Leu Asn
1490 1495 1500

Lys Leu Cys Arg Asp Glu Leu Cys Arg Cys Ala Glu Glu Asn Cys
1505 1510 1515

Phe Ile Gln Lys Ser Asp Asp Lys Val Thr Leu Glu Glu Arg Leu
1520 1525 1530

Asp Lys Ala Cys Glu Pro Gly Val Asp Tyr Val Tyr Lys Thr Arg
1535 1540 1545

Leu Val Lys Val Gln Leu Ser Asn Asp Phe Asp Glu Tyr Ile Met
1550 1555 1560

Ala Ile Glu Gln Thr Ile Lys Ser Gly Ser Asp Glu Val Gln Val
1565 1570 1575

Gly Gln Gln Arg Thr Phe Ile Ser Pro Ile Lys Cys Arg Glu Ala
1580 1585 1590

Leu Lys Leu Glu Glu Lys Lys His Tyr Leu Met Trp Gly Leu Ser
1595 1600 1605

Ser Asp Phe Trp Gly Glu Lys Pro Asn Leu Ser Tyr Ile Ile Gly
1610 1615 1620

Lys Asp Thr Trp Val Glu His Trp Pro Glu Glu Asp Glu Cys Gln
1625 1630 1635

Asp Glu Glu Asn Gln Lys Gln Cys Gln Asp Leu Gly Ala Phe Thr
1640 1645 1650

Glu Ser Met Val Val Phe Gly Cys Pro Asn
1655 1660

<210> 45
<211> 93
<212> PRT
<213> Homo sapiens

<300>
<308> Swiss-Prot/Q9H299
<309> 2003-02-28
<313> (1)..(93)

<400> 45

Met Ser Gly Leu Arg Val Tyr Ser Thr Ser Val Thr Gly Ser Arg Glu
1 5 10 15

Ile Lys Ser Gln Gln Ser Glu Val Thr Arg Ile Leu Asp Gly Lys Arg
20 25 30

Ile Gln Tyr Gln Leu Val Asp Ile Ser Gln Asp Asn Ala Leu Arg Asp
35 40 45

Glu Met Arg Ala Leu Ala Gly Asn Pro Lys Ala Thr Pro Pro Gln Ile
50 55 60

Val Asn Gly Asp Gln Tyr Cys Gly Asp Tyr Glu Leu Phe Val Glu Ala
65 70 75 80

Val Glu Gln Asn Thr Leu Gln Glu Phe Leu Lys Leu Ala
85 90

<210> 46
<211> 567
<212> PRT
<213> Homo sapiens

<300>
<308> Swiss-Prot/Q96RQ9
<309> 2003-02-28
<313> (1)..(567)

<400> 46

Met Ala Pro Leu Ala Leu His Leu Leu Val Leu Val Pro Ile Leu Leu
1 5 10 15

Ser Leu Val Ala Ser Gln Asp Trp Lys Ala Glu Arg Ser Gln Asp Pro
20 25 30

Phe Glu Lys Cys Met Gln Asp Pro Asp Tyr Glu Gln Leu Leu Lys Val
35 40 45

Val Thr Trp Gly Leu Asn Arg Thr Leu Lys Pro Gln Arg Val Ile Val
50 55 60

Val Gly Ala Gly Val Ala Gly Leu Val Ala Ala Lys Val Leu Ser Asp
65 70 75 80

Ala Gly His Lys Val Thr Ile Leu Glu Ala Asp Asn Arg Ile Gly Gly
85 90 95

Arg Ile Phe Thr Tyr Arg Asp Gln Asn Thr Gly Trp Ile Gly Glu Leu
100 105 110

Gly Ala Met Arg Met Pro Ser Ser His Arg Ile Leu His Lys Leu Cys
115 120 125

Gln Gly Leu Gly Leu Asn Leu Thr Lys Phe Thr Gln Tyr Asp Lys Asn
130 135 140

Thr Trp Thr Glu Val His Glu Val Lys Leu Arg Asn Tyr Val Val Glu
145 150 155 160

Lys Val Pro Glu Lys Leu Gly Tyr Ala Leu Arg Pro Gln Glu Lys Gly
165 170 175

His Ser Pro Glu Asp Ile Tyr Gln Met Ala Leu Asn Gln Ala Leu Lys
180 185 190

Asp Leu Lys Ala Leu Gly Cys Arg Lys Ala Met Lys Lys Phe Glu Arg
195 200 205

His Thr Leu Leu Glu Tyr Leu Leu Gly Glu Gly Asn Leu Ser Arg Pro
210 215 220

Ala Val Gln Leu Leu Gly Asp Val Met Ser Glu Asp Gly Phe Phe Tyr
225 230 235 240

Leu Ser Phe Ala Glu Ala Leu Arg Ala His Ser Cys Leu Ser Asp Arg
245 250 255

Leu Gln Tyr Ser Arg Ile Val Gly Gly Trp Asp Leu Leu Pro Arg Ala
260 265 270

Leu Leu Ser Ser Leu Ser Gly Leu Val Leu Leu Asn Ala Pro Val Val
275 280 285

Ala Met Thr Gln Gly Pro His Asp Val His Val Gln Ile Glu Thr Ser
290 295 300

Pro Pro Ala Arg Asn Leu Lys Val Leu Lys Ala Asp Val Val Leu Leu
305 310 315 320

Thr Ala Ser Gly Pro Ala Val Lys Arg Ile Thr Phe Ser Pro Pro Leu
325 330 335

Pro Arg His Met Gln Glu Ala Leu Arg Arg Leu His Tyr Val Pro Ala
340 345 350

Thr Lys Val Phe Leu Ser Phe Arg Arg Pro Phe Trp Arg Glu Glu His
355 360 365

Ile Glu Gly Gly His Ser Asn Thr Asp Arg Pro Ser Arg Met Ile Phe
370 375 380

Tyr Pro Pro Pro Arg Glu Gly Ala Leu Leu Leu Ala Ser Tyr Thr Trp

385 390 395 400

Ser Asp Ala Ala Ala Ala Phe Ala Gly Leu Ser Arg Glu Glu Ala Leu
405 410 415

Arg Leu Ala Leu Asp Asp Val Ala Ala Leu His Gly Pro Val Val Arg
420 425 430

Gln Leu Trp Asp Gly Thr Gly Val Val Lys Arg Trp Ala Glu Asp Gln
435 440 445

His Ser Gln Gly Gly Phe Val Val Gln Pro Pro Ala Leu Trp Gln Thr
450 455 460

Glu Lys Asp Asp Trp Thr Val Pro Tyr Gly Arg Ile Tyr Phe Ala Gly
465 470 475 480

Glu His Thr Ala Tyr Pro His Gly Trp Val Glu Thr Ala Val Lys Ser
485 490 495

Ala Leu Arg Ala Ala Ile Lys Ile Asn Ser Arg Lys Gly Pro Ala Ser
500 505 510

Asp Thr Ala Ser Pro Glu Gly His Ala Ser Asp Met Glu Gly Gln Gly
515 520 525

His Val His Gly Val Ala Ser Ser Pro Ser His Asp Leu Ala Lys Glu
530 535 540

Glu Gly Ser His Pro Pro Val Gln Gly Gln Leu Ser Leu Gln Asn Thr
545 550 555 560

Thr His Thr Arg Thr Ser His
565

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Met Ala Arg Val Leu Gly Ala Pro Val Ala Leu Gly Leu Trp Ser Leu
1 5 10 15

Cys Trp Ser Leu Ala Ile Ala Thr Pro Leu Pro Pro Thr Ser Ala His
20 25 30

Gly Asn Val Ala Glu Gly Glu Thr Lys Pro Asp Pro Asp Val Thr Glu
35 40 45

Arg Cys Ser Asp Gly Trp Ser Phe Asp Ala Thr Thr Leu Asp Asp Asn
50 55 60

Gly Thr Met Leu Phe Phe Lys Gly Glu Phe Val Trp Lys Ser His Lys
65 70 75 80

Trp Asp Arg Glu Leu Ile Ser Glu Arg Trp Lys Asn Phe Pro Ser Pro
85 90 95

Val Asp Ala Ala Phe Arg Gln Gly His Asn Ser Val Phe Leu Ile Lys
100 105 110

Gly Asp Lys Val Trp Val Tyr Pro Pro Glu Lys Lys Glu Lys Gly Tyr
115 120 125

Pro Lys Leu Leu Gln Asp Glu Phe Pro Gly Ile Pro Ser Pro Leu Asp
130 135 140

Ala Ala Val Glu Cys His Arg Gly Glu Cys Gln Ala Glu Gly Val Leu
145 150 155 160

Phe Phe Gln Gly Asp Arg Glu Trp Phe Trp Asp Leu Ala Thr Gly Thr
165 170 175

Met Lys Glu Arg Ser Trp Pro Ala Val Gly Asn Cys Ser Ser Ala Leu
180 185 190

Arg Trp Leu Gly Arg Tyr Tyr Cys Phe Gln Gly Asn Gln Phe Leu Arg
195 200 205

Phe Asp Pro Val Arg Gly Glu Val Pro Pro Arg Tyr Pro Arg Asp Val
210 215 220

Arg Asp Tyr Phe Met Pro Cys Pro Gly Arg Gly His Gly His Arg Asn
225 230 235 240

Gly Thr Gly His Gly Asn Ser Thr His His Gly Pro Glu Tyr Met Arg
245 250 255

Cys Ser Pro His Leu Val Leu Ser Ala Leu Thr Ser Asp Asn His Gly
260 265 270

Ala Thr Tyr Ala Phe Ser Gly Thr His Tyr Trp Arg Leu Asp Thr Ser
275 280 285

Arg Asp Gly Trp His Ser Trp Pro Ile Ala His Gln Trp Pro Gln Gly

290 295 300

Pro Ser Ala Val Asp Ala Ala Phe Ser Trp Glu Glu Lys Leu Tyr Leu
305 310 315 320

Val Gln Gly Thr Gln Val Tyr Val Phe Leu Thr Lys Gly Gly Tyr Thr
325 330 335

Leu Val Ser Gly Tyr Pro Lys Arg Leu Glu Lys Glu Val Gly Thr Pro
340 345 350

His Gly Ile Ile Leu Asp Ser Val Asp Ala Ala Phe Ile Cys Pro Gly
355 360 365

Ser Ser Arg Leu His Ile Met Ala Gly Arg Arg Leu Trp Trp Leu Asp
370 375 380

Leu Lys Ser Gly Ala Gln Ala Thr Trp Thr Glu Leu Pro Trp Pro His
385 390 395 400

Glu Lys Val Asp Gly Ala Leu Cys Met Glu Lys Ser Leu Gly Pro Asn
405 410 415

Ser Cys Ser Ala Asn Gly Pro Gly Leu Tyr Leu Ile His Gly Pro Asn
420 425 430

Leu Tyr Cys Tyr Ser Asp Val Glu Lys Leu Asn Ala Ala Lys Ala Leu
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Pro Gln Pro Gln Asn Val Thr Ser Leu Leu Gly Cys Thr His
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Met Asp Pro Arg Lys Val Asn Glu Leu Arg Ala Phe Val Lys Met Cys
1 5 10 15

Lys Gln Asp Pro Ser Val Leu His Thr Glu Glu Met Arg Phe Leu Arg
20 25 30

Glu Trp Val Glu Ser Met Gly Gly Lys Val Pro Pro Ala Thr Gln Lys
35 40 45

Ala Lys Ser Glu Glu Asn Thr Lys Glu Glu Lys Pro Asp Ser Lys Lys
50 55 60

Val Glu Glu Asp Leu Lys Ala Asp Glu Pro Ser Ser Glu Glu Ser Asp
65 70 75 80

Leu Glu Ile Asp Lys Glu Gly Val Ile Glu Pro Asp Thr Asp Ala Pro
85 90 95

Gln Glu Met Gly Asp Glu Asn Ala Glu Ile Thr Glu Glu Met Met Asp
100 105 110

Gln Ala Asn Asp Lys Lys Val Ala Ala Ile Glu Ala Leu Asn Asp Gly
115 120 125

Glu Leu Gln Lys Ala Ile Asp Leu Phe Thr Asp Ala Ile Lys Leu Asn
130 135 140

Pro Arg Leu Ala Ile Leu Tyr Ala Lys Arg Ala Ser Val Phe Val Lys
145 150 155 160

Leu Gln Lys Pro Asn Ala Ala Ile Arg Asp Cys Asp Arg Ala Ile Glu
165 170 175

Ile Asn Pro Asp Ser Ala Gln Pro Tyr Lys Trp Arg Gly Lys Ala His

180 185 190

Arg Leu Leu Gly His Trp Glu Glu Ala Ala His Asp Leu Ala Leu Ala
195 200 205

Cys Lys Leu Asp Tyr Asp Glu Asp Ala Ser Ala Met Leu Lys Glu Val
210 215 220

Gln Pro Arg Ala Gln Lys Ile Ala Glu His Arg Arg Lys Tyr Glu Arg
225 230 235 240

Lys Arg Glu Glu Arg Glu Ile Lys Glu Arg Ile Glu Arg Val Lys Lys
245 250 255

Ala Arg Glu Glu His Glu Arg Ala Gln Arg Glu Glu Glu Ala Arg Arg
260 265 270

Gln Ser Gly Ala Gln Tyr Gly Ser Phe Pro Gly Gly Phe Pro Gly Gly
275 280 285

Met Pro Gly Asn Phe Pro Gly Gly Met Pro Gly Met Gly Gly Gly Met
290 295 300

Pro Gly Met Ala Gly Met Pro Gly Leu Asn Glu Ile Leu Ser Asp Pro
305 310 315 320

Glu Val Leu Ala Ala Met Gln Asp Pro Glu Val Met Val Ala Phe Gln
325 330 335

Asp Val Ala Gln Asn Pro Ala Asn Met Ser Lys Tyr Gln Ser Asn Pro
340 345 350

Lys Val Met Asn Leu Ile Ser Lys Leu Ser Ala Lys Phe Gly Gly Gln
355 360 365

Ala

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Met Gln Leu Met His Ala Asn Ala Gln
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Leu Thr Leu Asp Ser Asn Thr Lys Tyr
1 5

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Phe Val Ile Asp Lys Ser Gly Ser Met
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Tyr Leu Leu Asp Ser Asn Ser Trp Ile
1 5

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Tyr Glu Leu Asp Lys Ala Phe Ser Asp
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Ile Lys Ser Gln Gln Ser Glu Val Thr
1 5

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Val Gln Ile Glu Thr Ser Pro Pro Ala
1 5

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Ile Ile Leu Asp Ser Val Asp Ala Ala
1 5

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Ile Glu Pro Asp Thr Asp Ala Pro Gln
1 5

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